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ENDEMIC ZOONOSES: A ONE HEALTH APPROACH

by

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**Submitted in fulfilment of the requirements for the
Degree of Masters by Research in Animal Health**

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ABSTRACT

Endemic zoonotic diseases remain a serious but poorly recognised problem in affected communities in developing countries. Despite the overall burden of zoonoses on human and animal health, information about their impacts in endemic settings is lacking and most of these diseases are continuously being neglected. The non-specific clinical presentation of these diseases has been identified as a major challenge in their identification (even with good laboratory diagnosis), and control. The signs and symptoms in animals and humans respectively, are easily confused with other non-zoonotic diseases, leading to widespread misdiagnosis in areas where diagnostic capacity is limited. The communities that are mostly affected by these diseases live in close proximity with their animals which they depend on for livelihood, which further complicates the understanding of the epidemiology of zoonoses. This thesis reviewed the pattern of reporting of zoonotic pathogens that cause febrile illness in malaria endemic countries, and evaluates the recognition of animal associations among other risk factors in the transmission and management of zoonoses. The findings of the review chapter were further investigated through a laboratory study of risk factors for bovine leptospirosis, and exposure patterns of livestock coxiellosis in the subsequent chapters.

A review was undertaken on 840 articles that were part of a bigger review of zoonotic pathogens that cause human fever. The review process involves three main steps: filtering and reference classification, identification of abstracts that describe risk factors, and data extraction and summary analysis of data. Abstracts of the 840 references were transferred into a Microsoft excel spread sheet, where several subsets of abstracts were generated using excel filters and text searches to classify the content of each abstract. Data was then extracted and summarised to describe geographical patterns of the pathogens reported, and determine the frequency animal related risk factors were considered among studies that investigated risk factors for zoonotic pathogen transmission. Subsequently, a seroprevalence study of bovine leptospirosis in northern Tanzania was undertaken in the second chapter of this thesis. The study involved screening of serum samples, which were obtained from an abattoir survey and cross-sectional study (Bacterial Zoonoses Project), for antibodies against *Leptospira* serovar Hardjo. The data were analysed using generalised linear mixed models (GLMMs), to identify risk factors for cattle infection. The final chapter was the analysis of Q fever data, which were also obtained from the Bacterial Zoonoses Project, to determine exposure patterns across livestock species using generalized linear mixed models (GLMMs).

Leptospira spp. (10.8%, 90/840) and *Rickettsia* spp. (10.7%, 86/840) were identified as the most frequently reported zoonotic pathogens that cause febrile illness, while Rabies virus (0.4%, 3/840) and *Francisella* spp. (0.1%, 1/840) were least reported, across malaria endemic countries. The majority of the pathogens were reported in Asia, and the frequency of reporting seems to be higher in areas where outbreaks are mostly reported. It was also observed that animal related risk factors are not often considered among other risk factors for zoonotic pathogens that cause human fever in malaria endemic countries. The seroprevalence study indicated that *Leptospira* serovar Hardjo is widespread in cattle population in northern Tanzania, and animal husbandry systems and age are the two most important risk factors that

influence seroprevalence. Cattle in the pastoral systems and adult cattle were significantly more likely to be seropositive compared to non-pastoral and young animals respectively, while there was no significant effect of cattle breed or sex. Exposure patterns of *Coxiella burnetii* appear different for each livestock species. While most risk factors were identified for goats (such as animal husbandry systems, age and sex) and sheep (animal husbandry systems and sex), there were none for cattle. In addition, there was no evidence of a significant influence of mixed livestock-keeping on animal coxiellosis.

Zoonotic agents that cause human fever are common in developing countries. The role of animals in the transmission of zoonotic pathogens that cause febrile illness is not fully recognised and appreciated. Since *Leptospira spp.* and *C. burnetii* are among the most frequently reported pathogens that cause human fever across malaria endemic countries, and are also prevalent in livestock population, control and preventive measures that recognise animals as source of infection would be very important especially in livestock-keeping communities where people live in close proximity with their animals.

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DECLARATION

I hereby declare that I am the sole author of this thesis; that the thesis is my own work and has never been previously accepted for a higher degree; and all references were duly consulted by me.

Signed.....

Divine Ejikeme Ekwem

Date.....

INTRODUCTION

Over the past 30 years, there has been increasing recognition of the importance and impact of zoonotic infections, with 61% of all human pathogens and 75% of emerging human infectious diseases classified as zoonotic (Taylor et al., 2001). Now, more than ever, there is a need for multidisciplinary and interdisciplinary approaches to enable the development of sustainable strategies to control and prevent these diseases. A collaborative agreement among all relevant human and animal health agencies would be critical (FAO-OIE-WHO, 2015).

While much international concern has focussed on emerging zoonotic pathogens that have epidemic and pandemic potential such as highly pathogenic avian influenza (HPAI) virus, severe acute respiratory syndrome coronavirus (SARS-CoV), and Ebola virus, less attention has been paid to endemic and neglected zoonotic agents (Mableson et al., 2014; Maudlin et al., 2009; Molyneux et al., 2011). A major reason that the impact of endemic zoonoses is poorly recognised, is that these diseases often lack a distinctive clinical presentation and laboratory diagnosis is challenging, particularly in resource-poor countries where laboratory capacity is weak (Halliday et al., 2015). Furthermore, in communities with poor access to health services, delays in healthcare-seeking behaviour are common and can result in chronic presentation of diseases, which can be very difficult to diagnose even with good laboratory facilities (Halliday et al., 2015). As a result, in many endemic settings, particularly impoverished communities, misdiagnosis of zoonoses is widespread resulting in poor patient management, a high disease burden and low awareness of the disease problem (Halliday et al., 2015).

Endemic zoonoses are associated with several major syndromes, including febrile illness, neurological syndrome and diarrhoea. However, there are no robust data in the literature to quantify the contribution of zoonotic diseases to these syndromes. Currently, there is increasing evidence that they are likely to contribute much more as a cause of febrile illness than widely recognised (Crump et al., 2013; Halliday et al., 2015). There are also growing calls for integrated 'One Health' approaches to the control of endemic zoonotic diseases with perhaps concerns that medical attention tends to be focussed mostly on the human population, thereby neglecting the primary source of the disease especially those from domestic animals (Day, 2011).

In the first part of this thesis, the opportunity of a review of zoonoses as causes of non-malaria fever in people was used to examine geographic patterns and frequency of reporting of animal-related risk factors from these publications. Guided by results from the review, I subsequently conducted a study of the seroprevalence on bovine leptospirosis and livestock coxiellosis in Tanzania in the second and third chapters of this thesis. Finally, I aim to synthesize results from these findings to make recommendations on the prevention and control of zoonotic pathogens and the diseases they cause.

CHAPTER ONE

Zoonoses, Human Febrile Illness and Animal Associations: a Review

1.1 INTRODUCTION

It is impossible to state exactly the first record of zoonotic diseases. Studies on historic data have suggested that the first zoonotic epidemic may have been recorded during the eighteenth century BC Babylonian era of the '*mad dogs*', which is most probably known today as rabies (Kruse et al., 2004). Similar reports were also made in the fourth century BC Talmud report of '*mad dog*' episodes, in 429-426 BC and 1320 and, the Bible recorded the first plague epidemic among the Philippians and then the 'plague of Athens' respectively (Baum, 2004). Thucydides the survivor of the plague described the symptoms as: fever, vomiting, headache, diarrhoea, facial erythema, bleeding, rashes that lead to ulcers (Baum, 2004). These signs are suggestive of a range of zoonotic infections observed today. Epidemiological scholars in that era tried to attribute the cause of these outbreaks of fevers to diseases that are currently known as typhus, smallpox, Rift Valley Fever, anthrax or dengue (Baum, 2004).

To trace the origin of zoonotic diseases from the very beginning, one would consider the relationship between humans and animals. In the pre-historic times, early man contact with animals was mainly associated with food source through hunting, preparation and consumption. Subsequent domestication of animals resulted in closer relationship between man and animals and greater availability of animal-source food at all times. Over several centuries, these relations have changed. Today, the long-standing relationship has now evolved to even more intimacy with huge benefits and risks to man. Animals are now being used for food, milk, clothing, pet companions, guards, recreational activities, research and learning. Notwithstanding the benefits humans have derived from the association, there is also a price to pay: infections and diseases called zoonoses.

Although the investment in emerging zoonoses is driven by pandemic threats and concerns of wealthier countries (Molyneux et al., 2011), the burden and impacts of zoonoses are still mostly felt in low and medium income countries (Aklilu, 2008). The burden relates to the direct public health impacts arising from zoonotic diseases in people, but also the indirect effects on human health, livelihoods and well-being that arise from zoonotic disease impacts on animals. Those zoonoses that cause production and performance losses in livestock, which

are critical for food security and income of millions of the world's poorest people, appear to exert the highest impacts in these endemic settings (Aklilu, 2008; Molyneux et al., 2011).

1.1.1 Definition of zoonoses

The expert working committee of the World Health Organisation and the Food and Agricultural Organisation, has defined zoonoses as those diseases and infections that are naturally transmitted from animals to humans (WHO, 1959). Zoonotic pathogens span a wide range of taxonomic groups, including bacteria, virus and *Rickettsia*, fungi, parasite and protozoa. Although the total number of zoonotic pathogens is probably still unknown, the study of Taylor et al., 2001, which reviewed secondary literature, reported 61% of the 1415 recorded human pathogens as zoonotic. The study also estimated that about 75% of emerging human infectious diseases are caused by zoonotic pathogens.

The need for control and prevention of zoonoses has been one of the driving forces behind the resurgence of interest in One Health approaches. One Health has been defined as all inclusive collaborative efforts of several disciplines such as medical, veterinary, other scientific, and social sciences working in local, national and global levels, towards the improvement of human and animal health, and the ecosystem (King, 2008). The One Health initiative movement is seen as a more holistic approach to foster zoonotic disease control by addressing infection in both animals and humans, and incorporate environmental, social and economic dimensions (Zinsstag et al., 2011). One Health strategy, however effective, appears to be more focused on emerging zoonotic threat because endemic zoonoses are still being neglected.

1.1.2 Transmission routes

As well as encompassing a broad taxonomic range of pathogens, zoonotic agents can also infect a wide range of host species and be transmitted by a variety of transmission routes, including direct and indirect contact, oral/ingestion, aerosol, bites and vector borne (Woolhouse et al., 2001). As a result, understanding reservoirs, sources of infection and risk factors for human infection can often be challenging.

Direct and Indirect contact: The direct transmission of zoonotic pathogens such as *Brucella* spp., *Leptospira* spp., *Mycobacterium tuberculosis*, and *Coxiella burnetii* has well been documented. For example, humans can acquire infections from these agents when in direct contact with infected animal materials like urine, milk, saliva, faeces and meat (Babudieri, 1959; McDermott and Arimi, 2002), and specifically, *Leptospira* spp. can be transmitted through direct contact with skin and mucous membranes (Wallach et al., 1997). Fomite transmission of these pathogens may also be common as an occupational hazard among animal handlers and abattoir workers, where personal and farm hygiene and biosecurity measures are often poorly implemented, particularly, in most developing countries where many zoonotic diseases are endemic.

Ingestion/Oral: This is the major route of transmission for zoonotic pathogens such as *Campylobacter* spp. and non-typhoidal *Salmonella* spp., and occurs when contaminated food, water, unpasteurised milk and improperly cooked animal products are ingested. For example, the consumption of unpasteurised milk has been implicated in the transmission of brucellosis (Corbel, 2006; Díaz-Aparicio, 2013), and bovine tuberculosis to humans (Atkins, 2000a, 2000b; Kazwala et al., 1998). In many traditional livestock-keeping communities in Africa and Asia, consumption of unpasteurised milk is widespread. More widely, demand for dairy products is increasing and dairy value chains become more complex, with implications for the transmission of milk-borne zoonoses. The consumption of improperly cooked goat meat has been identified as a risk factor for Q fever in Tanzania (Prabhu et al., 2011), while ingestion of faecal-contaminated food materials is considered the main transmission route for *Salmonella* spp. (Thorns, 2000). Poultry and poultry products have also been identified as a major source of bacteria food-borne zoonotic pathogens like *Campylobacter* spp. and *Salmonella* spp., which accounts for 90% of all related bacteria food-borne cases worldwide (Thorns, 2000).

Other zoonotic pathogens such as helminths, which are common in developing countries, are transmitted to humans after consumption of under cooked pork (Taeniasis, caused by *Taenia solium* or beef (*Taenia saginata*) (Gilman et al., 2012). In addition, the accidental ingestion of *Echinococcus* spp. eggs due to close contact with infective dogs can cause human hydatid disease that also occurs in low income countries (Robinson and Dalton, 2009).

Aerosol/ Inhalation: Zoonotic diseases can also be transmitted by inhalation through air droplets, sneezing and coughing. Anthrax, Avian influenza, Q fever, tularaemia, can all be

transmitted through aerosol. Exposures to their causative pathogens are common when aerosolised, and are transmitted as dust particles. A typical example was reported during the outbreak of Q fever in the Netherlands when large quantity of *C. burnetii* was shed in birthing materials of aborting goats, and human infection occurred through inhalation of the aerosolised bacteria (Hoek et al., 2010; Roest et al., 2011). Transmission can also occur when fluid, urine, and faeces from infected animals contaminate soil and when dried, can be inhaled as dust particles. Inhalation of anthrax spores directly from wool, hide and skin or aerosolized in the air is a common occupational hazard especially in endemic countries where routine anthrax livestock vaccination is not practiced (WHO, 2008).

Vectors: Vector-borne diseases account for more than 17% of all infectious diseases causing more than 1 million deaths annually (WHO, 2016a). Vectors are relevant in the transmission of zoonotic pathogens such as *Trypanosoma spp.* and *Borrelia spp.* Some (biological vectors) are directly involved in the life cycle of the pathogens such as tsetse fly involvement in the transmission of *Trypanosoma spp.* (MacGregor et al., 2011), while others are mainly mechanical vectors, for example tabanid fly transmission of *Francisella tularensis* (Petersen et al., 2009). The abundance of these vectors would significantly affect the prevalence of the disease they transmit. *Rickettsia spp.* (spotted fever) (Zhang et al., 2011), *Borrelia burgdorferi* (Lyme disease) and *Borrelia hermsii* (tick-borne relapsing fever) (Hojgaard et al., 2008), and *Francisella tularensis* (tularemia) (Gemechu et al., 2011), are all transmitted to humans through arthropod vectors. Trypanosomiasis (African sleeping sickness) is largely transmitted by tsetse fly (MacGregor et al., 2011), while rodents are mainly responsible for transmission of Hantavirus spp. to humans (Chrispal et al., 2010). Rift Valley fever virus, Japanese Encephalitis virus, West Nile Virus that cause acute encephalitis in humans are mosquito-borne viruses, which are transmitted to humans through mosquito bites from infected animals (Fever et al., 2014; WHO, 2016a).

Bites and scratches: Rabies, one of the oldest known zoonoses is transmitted to humans through bites or scratches from several hosts species such as dog, raccoons, bats cats and monkeys (Susilawathi et al., 2012). However, in Africa and many other tropical settings, the domestic dogs are considered the most important source of rabies virus to humans through bites from rabid dogs (Cleaveland, 1998; Warrell, 2008). Human cat scratch disease caused by *Bartonella henselae* can be transmitted through bites and scratches from infective cats, and domestic cats are regarded the largest reservoir of the pathogen (Carithers, 1985; Margileth, 1993).

1.1.3 Reservoirs of zoonotic pathogens

The understanding of animal reservoirs of zoonotic pathogens is critical for their control (Welburn et al., 2015). However, the concept of reservoirs is complex and identifying reservoirs can be challenging and expensive (Haydon et al., 2002). Several definitions of animal reservoir of infection exist, some of which are contradictory (Ashford, 1997; Swinton et al., 2002). In 2002, Haydon and others defined reservoirs of infection as single or multiple populations that are linked epidemiologically or environment where the pathogen can be permanently maintained and from which infection is transmitted to the described target population (Haydon et al., 2002). They argued further that the confirmation of a reservoir is only possible when infection in the target population is not maintained after the removal of all transmission opportunities between the target and non-target populations (Haydon et al., 2002). Key elements of this definition include the ability of the pathogen to persist, and to act as a source of infection. The detection of a pathogen in an animal population, by itself, provides insufficient evidence to demonstrate persistence of infection. Zoonotic and emerging pathogens infect a wide range of host species (Cleaveland et al., 2001; Woolhouse et al., 2001), and with several potential reservoir systems, many of which have not yet been fully characterised. While animals are, by definition, critical in the epidemiology of zoonoses, the precise role of different animal host species and populations as reservoirs, maintenance hosts, sources of infection and carriers can often be difficult to identify precisely (Haydon et al., 2002; Viana et al., 2015). Intervention studies such as vaccination and ring-fencing, and genetic characteristics of pathogens among others, have all been suggested as practical approaches in identify reservoirs of infections (Haydon et al., 2002). However, establishing a reservoir status of emerging zoonotic pathogens with rapid antigenic variation such as highly pathogenic avian influenza A virus is problematic (Parrish et al., 2015). Rabies virus is maintained in domestic dog reservoirs in most of sub-Saharan Africa and the elimination of infection through mass dog vaccination has resulted in elimination of the disease in some areas, confirming the role of domestic dogs as reservoirs.

While it has been possible to characterise reservoir systems for rabies in some areas, other zoonoses present a more complex challenge. For leptospirosis, the situation is particularly complex, with many different serovars and species of *Leptospira*, multiple host-pathogen associations and the potential for environmental persistence (Adler, 2001). It is difficult to identify reservoir hosts of endemic zoonotic pathogens such as *Leptospira spp.* in a region of

high endemicity. Domestic rodents, cattle and others ruminants, have been suggested to be the reservoirs of infection where the pathogen can jump across different animal species network and circulates freely within cattle, sheep and goats population (for further discussion see chapter 2).

Wild animals remain a major source of zoonotic pathogens and are likely to play an important role as reservoirs of many zoonoses. Small rodents and deer are considered the natural reservoirs of *Borrelia burgdorferi* that causes Lyme borreliosis in humans and are transmitted by tick vectors (Barbour and Fish, 1993). The rapid increase in incidence of Lyme borreliosis in the mid 90s in the north-eastern States of America was attributed to the rise of white-tailed deer population and *Ixodes spp.* as a result of increased reforestation activities (Barbour and Fish, 1993; Kruse et al., 2004).

The recent Ebola virus disease epidemic in West Africa was directly linked to wildlife as the origin of the epidemic. Even though the natural reservoirs of the pathogen remains unknown, bats have been strongly linked as possible reservoirs (Calisher et al., 2006). Bats can introduce the pathogen directly to human populations or through wild primates from which humans can potentially acquire infection when in contact with infective animal products during processing of bush meat for consumption (Calisher et al., 2006). The potential pandemic threat of Ebola virus becomes possible in secondary transmissions caused by close contact with infected patients, direct contact with infected blood, tissue, or body fluids (Jaax et al., 1995). Similarly, wild birds have been suggested to be the primary reservoir of highly pathogenic avian influenza A virus from where the domestic birds acquire infection, and their migratory abilities implicates them for the inter-epidemic occurrence in poultry (Parrish et al., 2015). While domestic birds can also be potential reservoirs because they are the main sources of human infection, there is still debate on whether they can permanently maintain the pathogen without wildlife sources (Causey and Edwards, 2008). The origin of the outbreak of severe acute respiratory syndrome (SARS) in China was never confirmed. SARS coronavirus (SARS-CoV) is thought to have uncertain animal reservoir (probably bats), that can potentially transmit the pathogen to civet cats and other animals. Current review studies suggest that the SARS coronavirus (SARS-CoV) may have undergone mutation and evolved into a new type that was responsible for the epidemic (Shi and Hu, 2008).

1.1.4 Endemic vs emerging zoonoses

Endemic zoonoses are classified as zoonotic diseases that are consistently present in the described populations or geographic regions at a certain level, occasionally described as ‘lingering’ zoonoses (WHO, 2015a). The examples of endemic zoonoses would depend on the region of endemicity which could be influenced by ecological characteristics of the disease; population demography or specific environmental conditions like flooding; and most probably, due to the intensity of research on the disease in the specific region that would increase their reporting (Webster et al., 2016). For instance, bacterial zoonoses such as leptospirosis, brucellosis, rickettsiosis and Q fever are highly endemic throughout sub-Saharan Africa (Allan et al., 2015; Dean et al., 2012; Vanderburg et al., 2014). However, there is an impression that they are reported more often in eastern Africa compared to western regions where the focus appears to be on Anthrax, African sleeping sickness, and non-zoonotic endemic diseases such as typhoid and malaria (Isere et al., 2015). These diseases are perceived to be of higher importance among healthcare workers thus, the reasons for greater attention and more recognition relative to others (Isere et al., 2015). Endemic zoonoses exert a disproportionate burden in low-income setting (Halliday et al., 2015; Molyneux et al., 2011). The knowledge and awareness about the presence of these diseases is usually poor in affected communities. Some other major features of endemic zoonoses are that government decision makers and politicians ignore their actual existence, and hardly recognised their significant impact on livestock productivity and human health (Halliday et al., 2015). Therefore, the diseases are under-reported and their true impact highly underestimated. There is little or no infrastructure towards their control, so the diseases remain endemic.

Emerging zoonoses are highly infectious, characteristically associated with outbreaks in an unpredictable and unprecedented pattern involving huge pandemic potential, which normally creates global panic (Liu et al., 2014). Emerging zoonoses occur in both developed and developing countries; they are associated with huge public awareness because they can spread rapidly with serious potential to cause heavy human health and economic losses (Liu et al., 2014). There are certain features required to classify a zoonotic disease as emerging. The WHO/FAO/OIE joint consultation on emerging zoonotic diseases that was held in Geneva, 2004, included these features by describing emerging zoonoses as those diseases that are ‘newly identified/newly evolved’ or ‘has been recognised in the past’ but now have a

‘sudden increased incidence’ or ‘expansion in geographical distribution, host/vector range’ (WHO-FAO-OIE, 2004). In addition to the classical definition by WHO, emerging zoonotic pathogens are highly transmissible and have very short infectious period. These enable them to quickly spread across regions, facilitated by global human movement (Liu et al., 2014).

Emerging zoonotic diseases such as Ebola virus disease, HPAI, and SARS, rightly fit the WHO definition. For example, SARS-CoV and HPAI virus have been previously recognised but, each time the pathogens re-emerged, they appear to have evolved into different forms as seen in the recent outbreak of SARS-CoV in China (Liu et al., 2014; Shi and Hu, 2008). Similarly, there are frequent mutations/high mutation rates in Hemagglutinin (H or HA) and Neuraminidase (N or NA) with each epidemic of HPAI A viruses (Suzuki, 2005). Once human-to-human transmission has been established, the spread of emerging zoonotic diseases can be very rapid across wide geographical regions and national boundaries (Liu et al., 2014), and that is when the wealthy nations start to panic, as seen in the outbreak of SARS-CoV in Asia. Early cases were reported in the Guangdong Province in November, 2002 and by March, 2003 the virus has spread to several other provinces in China, and in the neighbouring Hong Kong (Lam et al., 2003). The ability of the virus to transmit by air travel was identified as the major reason for pan-Asian spread because of the frequency of domestic flights within China and international flights from China to other countries in the region.

Air travel and other human movement networks were also implicated in the spread of Ebola across the West African states and it is considered an important factor for the pandemic potential of emerging zoonoses. The recent outbreak in the west African region was considered the largest and most complex (CDC, 2014), which may have been enhanced by policies that encouraged freedom of movement within the economic community of West African states (ECOWAS). Ebola was first reported in December 2013 in Guinea, at a village that borders Sierra Leone and Liberia. Within weeks the disease had already spread to Sierra Leone and Liberia, and eventually got into Nigeria at the later stages of the outbreak when an infected Liberian visited Nigeria by air travel (Alexander et al., 2015).

1.1.5 Specific challenges of neglected zoonoses

There are many factors driving the neglect of zoonotic diseases in low-income settings. A lack of knowledge and awareness of the disease burden in affected areas, which has

perpetuated a cycle of neglect among policy makers, has been identified as one of the main reasons why common zoonotic diseases are being neglected (Molyneux et al., 2011; Welburn et al., 2015). The poor quality of data on the impact of zoonoses is partly driven by diagnostic challenges (including poor clinical awareness of zoonoses and non-specific clinical presentation) and poor capacity for laboratory diagnosis, but also exacerbated in neglected and impoverished communities, which have little political voice and are poorly served by health-care facilities (Halliday et al., 2015, 2014; Molyneux et al., 2011). Despite potential disease control and prevention measures being available for many endemic zoonoses, both in human and animal populations, interventions have rarely been implemented in Africa and many of these ‘lingering’ zoonoses are being gradually neglected or even forgotten. However, there are encouraging signs that the cycle of neglect may be reversed such as the WHO/OIE/FAO tripartite commitments to global elimination of canine rabies by 2030.

Zoonotic diseases may be neglected as a consequence of the prioritisation required by the decision-making process of government in poor countries due to very limited resources. Governments in developing countries are more likely to channel their scarce resource towards the control of diseases of high mortality because of the perceived human impact, resulting in the poor recognition of endemic zoonoses that may not result in high mortality (Welburn et al., 2015). There is always the challenge on which disease to tackle and some of these decisions are based on the informed advice from international organizations such as the World Health Organization (WHO). For instance, the WHO Global Burden of Disease (GBD) project that measures the overall effects of diseases based on several data sources including disability-adjusted-life year (DALY), indicated that none of the well-known endemic zoonotic diseases such as brucellosis, leptospirosis and rabies are among the 20 leading causes of mortality and morbidity in sub-Saharan Africa between 2000-2012 (WHO, 2016b). This will obviously cause priority to be given to those diseases that are ranked highest based on WHO GBD criterion when government, international charities and foreign aids are partnering with developing countries in disease control projects.

The challenge of identifying reservoirs and source populations, which is critical for the design/development of appropriate intervention strategies for disease control and elimination, is exacerbated for neglected diseases where limited funding may be available for research. As has been explained earlier, understanding reservoirs of infections is critical for disease control (Welburn et al., 2015), however, intervention studies to identify and eliminate

reservoirs are often complex and expensive (Haydon et al., 2002), and may not be feasible or cost effective in poor settings because of the limitation of available resources.

Rapid rates of urbanization in sub-Saharan Africa may also be affecting patterns of zoonotic disease risk. Urbanisation may result in both increase and decrease in disease risk. For example, leptospirosis has been described as a paradigm for an urban health problem emerging as a consequence of the growth of slums (Reis et al., 2008). Conversely, human rabies deaths mainly occur in rural areas as a result of a high incidence of dog rabies and poor access to health services and life-saving post-exposure prophylaxis (Knobel et al., 2005). For instance, in most metropolitan cities where unaccompanied dogs are rarely seen, and the vaccination of domestic dogs well enforced, cases of rabies are not common or reported, while in rural areas where dogs vaccination are not enforced, the incidence is usually high, but under-reported (Bello et al., 2007). This usually posed a huge challenge in encouraging government of affected regions to intensify actions on rabies control because of the lack of information about the disease burden in marginalised communities in the rural areas. For political reasons, some countries in West Africa, most especially Nigeria, are claiming that the disease is nearly absent. However, it is a wrong perception because in reality rabies is highly neglected in the most affected communities with no political voice.

1.1.6 Syndromes and problems in identifying zoonotic pathogens

The clinical presentation of endemic zoonoses has been a challenge for both veterinary and human clinicians because of non-specific signs and symptoms that are easily confused with several other endemic non-zoonotic diseases (Halliday et al., 2015). For example, in humans endemic zoonoses are associated with non-specific symptoms such as fever, headache, weakness of joints or muscle pains, loss of appetite, which are also seen in non-zoonotic diseases, such as malaria and typhoid fever (Halliday et al., 2015). In endemic settings where diagnosis is based mainly on clinical signs, clinicians are likely to consider malaria and typhoid (that are perceived to be more prevalent) than zoonotic diseases in differential diagnosis (Crump 2013). In addition, societal influence and cultural perception among healthcare workers appears to be silent, but significant cause of poor recognition of zoonoses (Chandler et al., 2008). A survey that explored reasons malaria is being over diagnosed in Tanzania revealed a culture of encouraging the diagnosis of malaria among clinicians

(Chandler et al., 2008). It appears that there is a considerable influence from peers in the healthcare sector and even patients to recognise malaria, and a growing perception that the disease is relatively easy to diagnosed and patients' readiness to accept the clinician verdict, were responsible for over diagnosing malaria (Chandler et al., 2008). The consequences however, have been the under and missed-diagnosis of several other endemic diseases and poor patient outcome.

There is always a long list of differential diagnoses to be considered for common disease syndromes in endemic settings, and capacity to perform valid diagnostic test remains a challenge (Halliday et al., 2015). Consequently, unreliable diagnostic assessment occurs leading to inappropriate treatment, poor patient management and substantial increase in disease burden. A typical example was the study in northern Tanzania that investigated the aetiology of acute febrile illness, where malaria was diagnosed in 60.7% febrile patients, but was the actual cause of fever in only 1.6% (Crump et al., 2013).

In animals, non-specific presentations are also common and may be more challenging, with confirmatory laboratory diagnosis almost non-existent in poor countries (Halliday et al., 2015). For instance, leptospirosis, Q fever, and brucellosis that cause production losses in livestock, cannot be easily differentiated clinically (Halliday et al., 2015), leading to wide scale of misdiagnoses. Although there is an international system of reporting many of these zoonoses, such the world animal health information system of the OIE (OIE WAHIS), for many parts of the developing world data are scant, incomplete and likely to be unreliable. For example, no data were shown for leptospirosis in certain areas in Africa on WAHIS maps, but when compared with information reported in recent systematic reviews (Allan et al., 2015), it appears there were publish papers on the disease during that same period. This indicates that the recommendation from the OIE to report these diseases is probably not being adhered to because of specific challenges in the identifying and reporting of diseases in developing countries. A review of the reasons for lack of surveillance on zoonotic diseases, also reported that insufficient funding, inadequate staffing, and inappropriately trained personnel were the major constraints in West Africa (Nigerian Federal Ministry of Health, 2007).

When Ebola virus was ravaging West African countries, it took several weeks for local health care professional to recognise the grave danger of the situation because they could not establish the reason for sudden haemorrhagic syndromes and death. There were no facilities

to test or quarantine affected individuals. Also, several zoonotic pathogens such as *Brucella* spp. and *Leptospira* spp. have variations in strains and types (Gemechu et al., 2011; Picardeau, 2013); molecular typing is required to identify circulating types to fully understand the epidemiology of the diseases they caused and to then strategize prevention and control in both human and animal populations. Without the facilities to adequately undertake these procedures, identifying and reporting these pathogens will remain a challenge in low and middle-income countries.

1.1.7 Economic significance

Economic impacts of emerging diseases are huge but the mortality is relatively low as compared to endemic zoonoses. The usual global panic associated with emerging zoonoses often masks the actual number of deaths compared to diseases that are always present. However, global awareness for most endemic zoonotic disease appears to be on the increase in low resources countries compare to previous years.

The globalisation and advancement in transport technology for instance, has reduced the whole wide world to a global village, thereby increasing movement of humans and animals. With the continuous movement throughout the globe and changes in vector biology due to social and environmental factors, zoonotic diseases have continuously re-emerged, with several inter-epidemic cases, and most of them eventually become endemic (Friend et al., 2006). Pandemic potential of emerging zoonoses heightened by globalization, and the economic consequences, remains a serious concern for wealthy nations and the main reasons for investment in tackling emerging zoonoses. For example the rapid spread of SARS-CoV in Asia was a huge concern for China (Lam et al., 2003; Shi and Hu, 2008). The epidemics have been very devastating, causing huge human and economic losses within a significant short period. The impact on affected countries during the outbreak of SARS-CoV in Asia was colossal (Liu et al., 2014). There were reductions in travelling to these affected regions and a significant drop in tourism (Lam et al., 2003). Likewise, the Ebola virus disease situation in West Africa created serious panic in the United States and Europe because of the consequences of a potential pandemic spread (CDC, 2014). The impact of Ebola is still being felt in affected countries in West Africa at both national and household levels. A recent survey conducted by World Bank Group, in partnership with Innovations for Poverty Action,

reported massive disruptions in the economy following national lockdown, food insecurity, and significant drop in household level income and livelihood (The World Bank, 2015).

Endemic zoonoses often have several impacts relating to both human and animal factors. The lack of integrated measures of disease burden is considered an important factor in the neglect of many endemic zoonoses (Molyneux et al., 2011), as the societal/overall burden of zoonotic diseases is often poorly reflected by a single standardised measure, such as the disability-adjusted-life-year (DALY). The DALY is commonly used to prioritise interventions by the public health sector and a useful framework for assessing economic impacts of zoonoses, but with main emphasis on human health (Carabin et al., 2005). Estimating the true impact of endemic zoonoses in terms of production and performance losses in animals and the burden on human factors such as mortality and morbidity, and other criteria of DALY is still not well developed for endemic zoonotic diseases in low resource country (Grace et al., 2012). To date, there is no reliable information about the actual burden of these common diseases (Grace et al., 2012) (See Chapter Two and Three for further details on the impacts of endemic zoonoses for specific pathogens).

1.1.8 Rationale and aims

This study used the opportunity of a systematic review of zoonotic causes of human febrile illness to conduct a nested review of the most frequently reported zoonoses to examine geographic patterns of disease, patterns of co-occurrence, and risk factors for human disease.

The key questions addressed in this review are:

1.0 What is the frequency of reporting of zoonotic pathogens that cause fever?

- Does the frequency of reporting have a geographical pattern?
- Can we confirm cases of concurrent infection of two or more pathogens?

2.0 To what extent are the contributions of animal hosts described and recognised in the literature on zoonotic causes of fever?

- Can we identify risk factors for human infections/host range of animal species in this literature?
- Is there evidence of specific host/pathogen associations in this literature?

1.2 METHODOLOGY

This review was conducted as part of a larger review project to investigate the contribution of zoonotic pathogens to febrile illness. The methodology of the larger review project is described in Box 1 and further details are provided in (Halliday et al., 2014).

Box 1: Methods used in the overall zoonoses and fever review.

We constructed three search concepts for 'Fever', 'Zoonoses' and 'Malaria Endemic Countries' and queried MEDLINE and EMBASE databases for references published in the period 2004 – 2012 that met the criteria of all three concepts. For the 'Zoonoses' concept we compiled a list of included pathogens based on one of the following three selection criteria:

- *Inclusion on the WHO list of Zoonoses and Veterinary Public Health listed diseases*
 - *Inclusion on the list of Zoonotic potential criterion of OIE listed diseases(2012) (excluding fish, bee, mollusc, crustacean and amphibian diseases)*
 - *Identification as a frequently reported zoonotic cause of human fever in the published literature based on preliminary searches using the search syntax "(exp Fever/ OR fever.mp.) AND (exp Zoonoses/ OR zoonoses.mp OR zoonosis.mp)" limited to humans.*
- Classification of the zoonotic status of each pathogen identified for search concept compilation and in references obtained was based on classifications made in a previous review of human pathogens. The 'Malaria Endemic Countries' concept was constructed using the list of 108 countries in which malaria has been classified as endemic by the WHO over the period 1990 to 2011.*
- AND the appendix of Taylor et al., 2001, that classified zoonotic status of all human pathogens.*
- Abstract and full-text evaluation were conducted by two independent reviewers following study defined inclusion and exclusion criteria in accordance with PRISMA guidelines (Moher et al., 2009).*

The review conducted for this study used the dataset of 840 articles identified by the search strategy described above in Box 1. The methodology for the review and analysis of these articles involves three main steps (Figure 1):

- 1- Filtering and reference classification
- 2- Identification of abstracts that described risk factors
- 3- Data extraction and summary analysis of data.

The abstracts of all references were transferred into a Microsoft Excel spreadsheet, where several subsets of abstracts were generated using Excel filters and text searches to classify the content of each abstract.

1.2.1 Filtering and reference classification

The abstracts of the 840 full-text articles were manipulated in an Excel spreadsheet. A list of 10 common zoonotic pathogens (Table 1) was selected based on a prior procedure of quantifying the frequency of reporting in a trial dataset of references that came up in a search for fever and zoonoses (Halliday et al., 2014). An Excel filter tool was then used to create a subset of abstracts that refer to these 10 common zoonotic pathogens or their synonyms (Table 1). The majority of the synonyms were identified in the articles used for the review. In order to identify synonyms that may not have been indexed in Medline, an additional search for each pathogen was carried out using the internet browser 'Google'. This enabled the identification of words or statements relating to the zoonotic pathogen, which have not been indexed, but may have been mentioned as a local name or term used by the scientific community when referring to the pathogen. The references from the 'Google' search were manually screened to identify other words or phrases that have been used in the articles reviewed to describe the pathogen. After identifying key words and possible synonyms for each pathogen, a search query was developed by a Boolean combinatory 'OR' of the key words and synonyms. This was done for all the 10 pathogens. For example, the query developed for *Rickettsia spp.* was 'Rickettsia' (key word), and 'Rickettsiae, Rickettsiosis, Typhus, Spotted fever, Orientia tsutsugamushi (Scrub typhus)', as synonyms. Using an Excel filter tool, the search queries were used to identify all abstracts in the dataset that included either the keyword and/or any of the listed synonyms. The procedure was repeated for each pathogen and all identified abstracts were recorded.

Table 1: List of the 10 Pathogens and their synonyms

Key search term relating to the pathogen	Additional search team
<i>Leptospira</i>	Leptospirosis, Leptospire, Rat fever
<i>Coxiella</i>	Q fever, <i>C. burnetti</i>
<i>Brucella</i>	Brucellosis, Undulant fever, Malta fever, Rock fever
<i>Rickettsia</i>	Rickettsiae, Rickettsiosis, Typhus, Spotted fever, <i>Orientia tsutsugamushi</i> (Scrub typhus)
Hantavirus	HPS, Pulmonary syndrome
West Nile Virus	WNV
<i>Borrelia</i>	Borreliosis, Lyme, (Lyme disease), Tick-borne relapsing fever (TBRF), Louse-borne relapsing fever (LBRF)
<i>Francisella</i>	<i>F. tularensis</i> , Tularemia, Tularaemia, Rabbit fever
<i>Salmonella</i>	Salmonellosis, non-typhoidal <i>Salmonella</i> (NTS)
Rabies virus	Rabies, Rabid

Manual screening

The abstracts that mentioned a zoonotic pathogen were manually screened to confirm investigation of a zoonotic pathogen. This process also enabled classification of abstracts, identification of abstracts that described risk factors and those that specifically referred to an animal group. The outcome of the manual screening was recorded with a further column created for each animal species. To validate the Excel filter process, 20% of randomly selected abstracts from the pool that did not mention a zoonotic pathogen were manually screened for confirmation. The manual screening procedure was also used to remove duplicate filtered abstracts.

Abstract classification

The second part of the first step involved classification of abstracts with confirmed pathogen inclusion. Abstracts were grouped based on whether the pathogen was the target of the study or mentioned in a more general context. The abstracts relating to studies that specifically investigated these zoonotic pathogens were further classified into three major categories: (a) country and geographical regions where the study was conducted; (b) journal of publication; (c) type of study design.

(a) Country and geographical regions where study was conducted

The country where the study was conducted was identified for each abstract. The main review focused on malaria endemic countries, which are located largely in Africa, Asia and Latin America and the Caribbean continents (WHO, 2015b). Countries were then grouped into regions and continents based on the current United Nations (UN) groupings (United Nations, 2016). At this stage, a further manual screening was carried out because, although the original inclusion criteria were for studies conducted in malaria endemic countries, several studies were included among the abstracts that had involved travellers who developed the zoonotic disease on returning to their home (non-malaria endemic) country. For these studies, the geographic region was classified according to the country where people had acquired the infection.

(b) Journal of publication

The journal of publication was classified into: Medical, Veterinary or Life Sciences based on the main theme of the journal. However, there are limitations in this classification because some journals may have more than one theme: specialist and general sections. For example, the journal of clinical microbiology (with life sciences as the main theme), which also has a dedicated section for veterinary related publications, would be classified under the main theme.

Medical Journal: This includes articles that were published in the journal of general medicine and journals of specialist medicine.

Veterinary Journal: This includes articles published in the veterinary and other allied animal health specialties.

Life Sciences: This includes journals of general life sciences, and others that could not be classified as medical or veterinary.

The full list of journals where these studies were published, and the list of countries where zoonotic pathogens were reported are attached in the appendix.

(c) Type of study design

To allow examination of risk factors, abstracts were classified into the following study designs: case-series, cohort study, case-control, and cross-sectional study. The remaining studies, which included mainly diagnostic test evaluation, randomized controlled trials,

clinical trials and experimental studies were grouped together as a separate category ('Other'). These classifications were assigned on the basis of how the study was described by the authors in the abstract (Table 2). By definition and concept, case-control studies, cohort studies and cross-sectional studies, are expected to consider risk factors for zoonotic disease transmission and were further screened for animal related risk factors.

Table 2: Classification of abstracts by study design

Case-series	A case series represents an observational study that reports data from a subject group without a comparison population (Dekkers et al., 2014; Vandenbroucke et al., 2007). In this review, case-series information was derived mostly from cases in health care settings, with all included individuals having the same case definition. Clinical cohorts where the study population was followed to allow sufficient time for clinical and laboratory diagnosis were classified in this category.
Cohort study	The reviewed abstracts with cohort study design describe a study population with similar exposure risks (e.g. sampled based on living in a rural area, agriculture workers, cleaners etc) and were followed over time starting from a baseline point. Those that developed febrile illness and other related symptoms of disease were further investigated for aetiology and relevant risk factors for zoonotic diseases and absolute risks calculated (Dekkers et al., 2014; Vandenbroucke et al., 2007).
Cross-sectional study	This group contains abstracts with descriptive and analytical study designs where demographical information of the sampled febrile population were collected at a defined time as detail of the cause of the febrile illness for each recruited subject. This category includes abstracts that describe cause of fever, source of exposures and risk factors, prevalence and/or burden of zoonotic pathogens in the febrile population.
Case-control study	This classification include abstracts where febrile population with specific case definition for the investigated disease was compared with a suitable control group without the zoonotic disease, but would have been included as cases if they had. The associations or risk factors for the disease between the two groups were assessed.
Other	For the RCT abstracts, each subject in the sampled febrile population was randomly allocated to a particular group (treatment being evaluated group or control/group with alternative treatment). Studies that compared the sensitivity and specificity and effectiveness of different diagnostic methods to identify zoonotic pathogens in a febrile population were included in this group and other types of study not classified above.

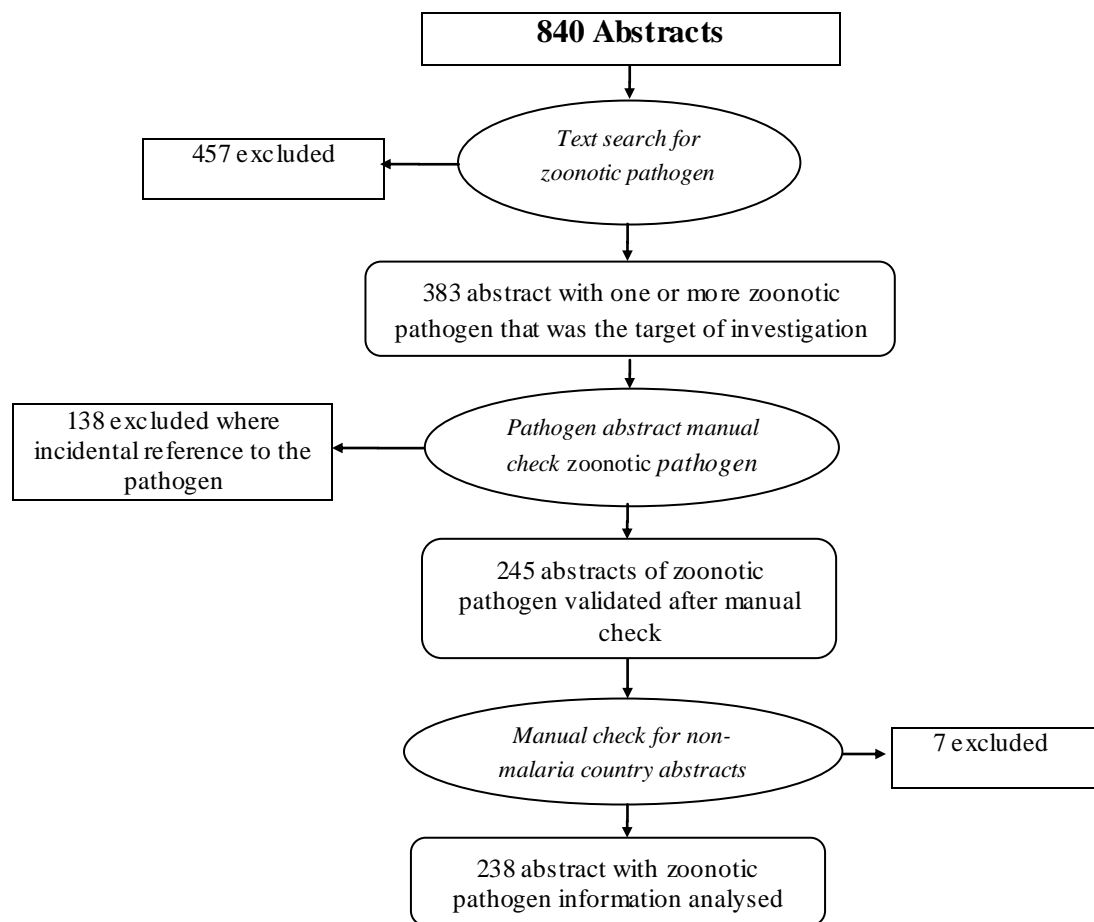
1.2.2 Risk factors for transmission and animal associations

The classification of abstracts by study design allows the identification of a subset of abstracts describing studies that were designed to identify risk factors for zoonotic disease transmission. This then enabled description of how often animal-related risk factors were considered within the subset of studies that considered risk factors. Studies that do not consider risk factors at all would not be expected to consider animal associations as risk factors for zoonotic disease transmission and these were therefore excluded from this analysis. For example, while case-control and cohort studies are designed to consider risk factors, case series and RCT are not (Table 2).

Building on the study design classification, a set of abstracts where the study investigated or considered risk factors for zoonotic disease transmission was created by manually screening all abstracts with case-control, cohort, and cross-sectional study designs (e.g. all abstracts classified as case-control, cohort, and cross-sectional designs were then manually screened and classified to indicate if they did or did not include content on any risk factors). Within the set of abstracts that did describe any risk factors, manual screening also created a subset that specified or mentioned animal associations as risk factors or source of the zoonotic pathogen investigated.

Abstracts were classified as having considered the role of animals if the study had either (a) specifically described the potential role(s) of animal species in transmission (i.e. all types of transmission including food-borne) to the human cases in the study or (b) had identified or quantified animal-related factors as specific risk factors or sources of infection for the human cases. Abstracts were excluded when (c) abstracts had mentioned animal species, but not in the context of the zoonotic pathogen or (d) animals were described in very general terms as being associated with the zoonotic pathogen but not discussed specifically in the context of the study.

Figure 1: A flow chart of events showing steps in the selection of abstracts



1.2.3 Full text review of *Leptospira spp.*

A full text review of papers that investigated *Leptospira* and described animal related risk factors was undertaken to allow an in depth understanding of potential variation in the reporting of zoonotic pathogens that cause human fever across malaria endemic countries. A further step of data extraction was performed on articles that investigated animal associations in relation to *Leptospira spp.*, the most frequently reported of the zoonotic pathogens.

Relevant aspects of strengthening the reporting of observational studies in epidemiology (STROBE Statement) (Vandenbroucke et al., 2007), and the pocket guide on critical appraisal (Crombie, 1996) were adopted and used to evaluate the methodological procedures on the pattern of reporting of the zoonotic pathogens, the overall quality of each paper reviewed, and to develop a systematic process for extracting data of this kind. A summary of each paper was made based on these evaluation criteria. Using these criteria, data were extracted from the *Leptospira* papers and were recorded on a specifically designed abstraction form. This process was done in a systematic, step- by- step manner which follows the same sequence for each paper, in order to avoid bias and to maintain consistency. The key elements extracted were:

- Title of the paper
- Study design
- Geographical settings of the study
- Nature of animal contact
- Type of study (occupational/non-occupational/outbreak)
- Animal species that was mentioned/associated with the pathogen
- Year and journal of publication
- Summary of result or main findings

1.3 RESULTS

1.3.1 Summary of pathogen abstracts

In total, 383 of 840 (45.6%) of the abstracts in the full data set that mentioned at least one of the 10 selected zoonotic pathogens and were retained (Figure 1). The manual screening of the 383 abstracts confirmed 29.2% (245/840) had actually investigated at least one of the pathogens. After the exclusion of the seven studies that were reported in non-malaria endemic countries, only 28.3% (238/840) papers remained. Summary analyses for the 10 zoonotic pathogens that were considered show that *Leptospira* spp. (10.8 %, 91/840) and *Rickettsia* group (10.7 %, 90/840) were the most frequently reported pathogens, while Rabies virus (0.4 %, 3/840) and *Francisella* spp. (0.4%, 1/840) were the least. The complete details of the number of abstract filtered for each pathogen and subsequently confirmed by manual screening are shown in Table 3.

Table 3: The number of abstracts filtered for each pathogen and proportion of the total abstract set that include investigation of this pathogen (NB: The total number of abstracts with confirmed pathogen information add up to more than 238 because some abstracts reported more than one zoonotic pathogens)

Pathogen group	Number of abstracts	% of abstracts (n=840)
<i>Leptospira</i>	90	10.8
<i>Rickettsia</i>	86	10.7
<i>Brucella</i>	37	4.4
Non-typhoidal <i>Salmonella</i>	29	3.5
<i>Coxiella</i>	24	2.9
Hantavirus	16	1.9
<i>Borrelia</i>	10	1.2
West Nile Virus	10	1.2
Rabies	3	0.4
<i>Francisella</i>	1	0.1

NB: *Rickettsia* group includes: Typhus fever, Spotted fever and Scrub typhus; *Borrelia* group includes: Lyme disease, Louse-borne relapsing fever and Tick-borne relapsing fever.

1.3.2 Characteristics of excluded abstracts

There were three stages of abstract selection and for each stage certain numbers of abstracts were excluded. The first was at the abstract filtering stage where 54.4% (457/840) of abstracts were excluded because they did not mention any of the 10 selected zoonotic pathogen (Figure 1). At the second stage, 138 abstracts that did not investigate a zoonotic

pathogen after manual screening confirmation were removed (Figure 1). This group of abstracts had described the pathogen in general terms but not specifically investigated in the context of the study. At the final stage of exclusion, seven of 245 remaining abstracts were excluded because the studies were conducted in non-malaria endemic countries and it was not categorically stated that the febrile population has a recent history of travelling to a malaria endemic country. These studies had considered the pathogens identified to be endemic in the non-malaria country where the study was conducted, and had only referred to a malaria endemic country as where the pathogen is known to be prevalent. A total of four of these were reported in Europe and *Coxiella burnetii* (1 abstract) and *Borrelia burgdorferi* (3 abstracts) were the pathogens investigated. For the other three, one reported West Nile Virus in Canada, while the remaining two reported *Rickettsia spp.* in Russia. Overall, 71.7% (602/840) abstracts were excluded from the analyses, leaving 238 abstracts for ongoing analyses (Figure 1).

Additional analyses described below were performed using the 238 abstracts with confirmed zoonotic pathogen information as the denominator (e.g. abstracts describing investigation of one of the 10 selected study zoonoses). This enabled comparison of the attributes of studies within this population of studies that describe investigation of one or more study zoonoses.

1.3.3 Co-occurrence of infection in study population

A total of 19.8 % (47/238) abstracts (with confirmed zoonotic pathogen information) reported studies where more than one zoonotic pathogen were investigated in the same study febrile population. The pathogens that were most commonly investigated in concurrent infections at the study population levels were *Leptospira spp.*, *Rickettsia spp.*, *Brucella spp.*, *Coxiella spp.* and non-typhoidal *Salmonella spp.* The number of abstracts reporting investigation of each pairwise combination of pathogens is given in Table 4. The concurrent investigation of *Leptospira spp.* and *Rickettsia spp.* occurred most often in 9.7 % (23/238) of the abstracts that investigated a zoonotic pathogen. The second highest incidence of concurrent investigation was *Rickettsia spp.* and *Coxiella spp.*, where 5.9 % (14/238) of abstracts investigated both pathogens in the sampled febrile population.

Table 4: Summary of abstracts in which more than one zoonotic pathogen were investigated in the study population

	Leptospira	Rickettsia	Brucella	NTS	Coxiella	Hantavirus	Borrelia	WNV
<i>Leptospira</i>	-	24	8	4	7	4	1	2
<i>Rickettsia</i>		-	6	6	14	3	4	2
<i>Brucella</i>			-	4	6	1	1	0
Non-typhoidal <i>Salmonella</i>				-	1	1	1	0
<i>Coxiella</i>					-	1	2	1
Hantavirus						-	1	1
<i>Borrelia</i>							-	0
West Nile Virus								-

1.3.4 Regional reporting of zoonotic pathogens across malaria endemic countries

The number of abstracts reported varies by regions. At least one publication was recorded from every region of every continent. *Leptospira spp.* and the *Rickettsia spp.* were mostly reported in Asia, while non-typhoidal *Salmonella spp.* were reported more in Africa than any other region (Table 5). Overall, zoonotic pathogens that cause human fever in this review were reported in 50 malaria endemic countries across the three continents. More were reported from Asia (55.0%, 131/238) in 20 countries, with the majority of studies (102 abstracts) reported in southern and southeast Asia (Figure 2). In Africa, 30.3 % (72/238) of abstracts were reported from 17 countries, with the highest number in the East African region (Figure 3). Latin America and the Caribbean had the smallest number 14.7% (35/238) of abstracts and the majority of these were reported in the southern region, with the least reported in Caribbean and central regions (Figure 4).

Figure 2: Proportion of abstracts that reported zoonotic pathogens across Asia region (n=131)

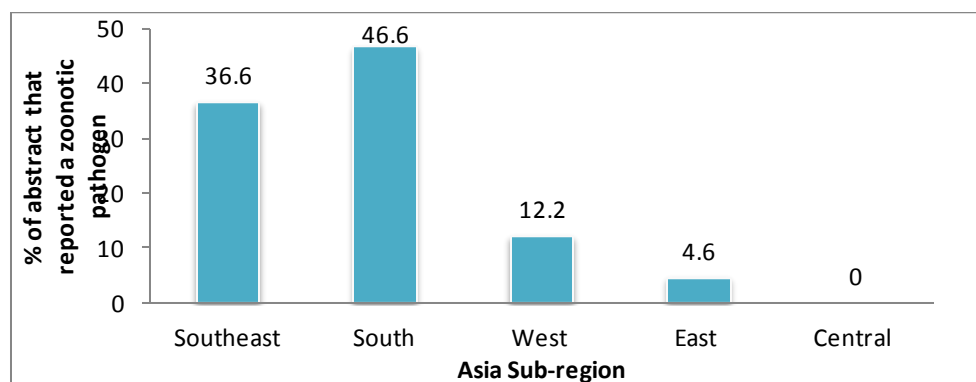


Figure 3: Proportion of abstracts that reported zoonotic pathogens across Africa region (n=72)

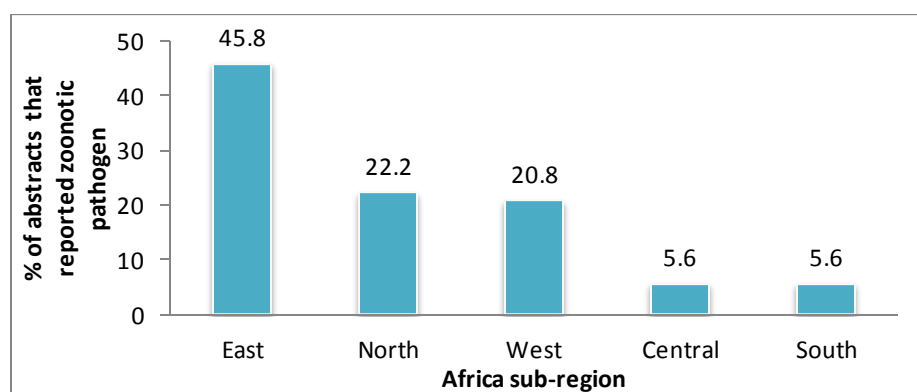


Figure 4: Proportion of abstracts that reported zoonotic pathogens across Latin America and the Caribbean (n=35)

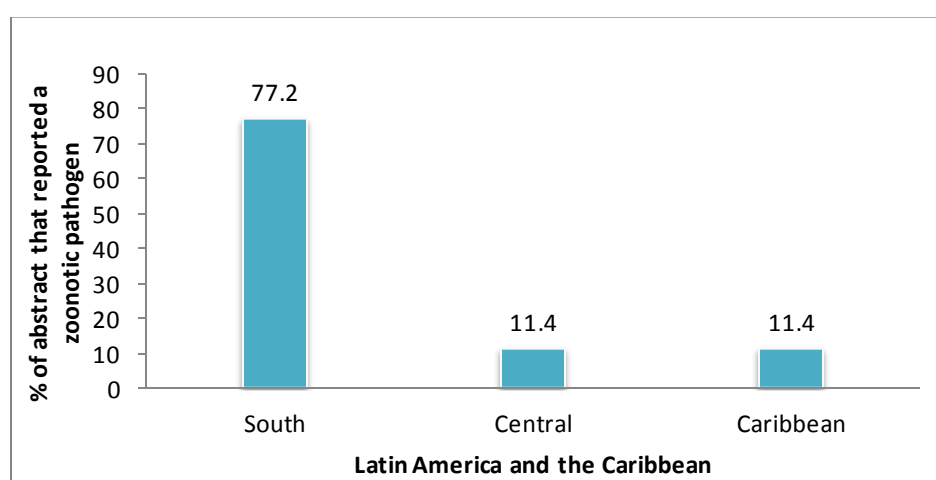


Table 5: Number of abstracts for each pathogen stratified by region

Pathogen group	Asia	Africa	Latin America and the Caribbean
<i>Leptospira</i>	60	10	20
<i>Rickettsia</i>	51	25	10
<i>Brucella</i>	20	14	3
Non-typhoidal <i>Salmonella</i>	5	22	2
<i>Coxiella</i>	14	6	4
Hantavirus	9	1	4
<i>Borrelia</i>	3	6	1
West Nile Virus	6	3	1
Rabies	1	2	0
<i>Francisella</i>	1	0	0

1.3.5 Classification of abstracts by journal of publication, study design and animal related risk factors

Journal of publication

Abstracts were considered in three major journals: medical related journals (52.9%, 126/238) life sciences (45.8%, 109/238) and veterinary (1.3%, 3/238).

Study design and Risk factors

All abstracts with pathogen information were case series (134), case control (36) cross sectional study (32), cohort study (8), and others (24) (Table 6).

Table 6: Classification of abstracts based on study design, risk factors and animal related risk factors

Sub-category	Number of Abstracts (%)	Number with Risk Factors	Number with animal related Risk Factors
Study design			
Case series	140 (58.8)	0	-
Case control	36 (15.1)	36	15
Cross sectional	32 (13.5)	7	4
Cohort study	2 (0.8)	2	1
Others*	28 (11.7)	0	-

*Include abstracts of studies that were design to specifically evaluated different diagnostic test, RCT, and other clinical trials

The manual screening of abstracts of case-control study, cohort and cross-sectional study designs, which are expected to consider risk factors, reported 45 abstracts addressed risk

factors for zoonotic disease transmission in the sampled febrile population. Overall, 44.4% (20/45) of these considered animal related risk factors (Table 6).

Several animal species were reported (Table 7). While some abstracts made mention of specific animal groups (e.g. dogs or cattle), others referred to general animal groups such as ‘livestock’ or ‘wildlife’.

Table 7: List of all terms used to refer to domestic animals and wildlife in the screened abstracts

Animal group	Other terms used
Cattle	Cow, Bull, Ox, Bovine, livestock,
Horse	Equine
Dog	Canine
Cat	Feline,
Rodent	Rat, Mouse, Mice,
Sheep	-
Goat	-
Bat	-
Bird	Poultry
Rabbit	-
Wildlife	Buffalo, Agouti paca
Animals	-

1.3.6 Cross tabulation

The cross matching of each of the 10 zoonotic pathogens that were frequently reported with or without their animal associations were applicable are shown in Table 8. Amongst the articles that mentioned one or more pathogen, the most frequently reported animal group was rodents (18 articles). The pathogens rodents were mostly associated with are *Leptospira* (8), *Rickettsia* (4) and *Hantavirus* (5). The animal group dog was also commonly associated with *Rickettsia spp.* while there was no clear pattern in the other animal groups and zoonotic pathogens (Table 8). All abstracts that investigated risk factors for *Borrelia spp.* and West Nile Virus considered animal associations, while none of the non-typhoidal *Salmonella spp.* abstracts considered animal related risk factors (Table 8).

Table 8: Zoonotic pathogens reviewed and the proportion of abstracts that associated the pathogen with animal groups (NB: the sum of no of abstracts adds up to more than 45 because some abstracts mentioned more than one pathogen)

Pathogen group	Number of Abstracts that Considered Risk factors	Number of abstracts with Animal related Risk factors	% abstracts with animal and zoonotic pathogen information
<i>Leptospira</i>	18	7	38.9
<i>Rickettsia</i>	15	3	20.0
<i>Brucella</i>	9	4	44.4
Non-typhoidal <i>Salmonella</i>	1	0	0.0
<i>Coxiella</i>	6	4	20.8
Hantavirus	3	1	33.3
<i>Borrelia</i>	2	2	100.0
West Nile Virus	1	1	100.0

1.3.7 *Leptospira* spp. and animal group

The zoonotic pathogen *Leptospira* spp. emerged as the most frequently reported pathogen based on the outcome of the pathogen classification, and the cross tabulations of pathogens and animal groups indicated it was most associated with animals. The seven abstracts that associated *Leptospira* spp. with animals mostly considered rodents and livestock related risk factors. There were five abstracts with reference to rodent groups, two with livestock (with an additional study that mentioned livestock and rodents), and one abstract mentioned wildlife groups (Table 9).

Full texts of the seven *Leptospira* group abstracts with animal information that were reviewed in detail are shown in Table 9. The nature of animal related risk factors described depends on the regions where the study was undertaken. Those reported in Asia mainly investigated outbreaks in communities where rice and livestock farming were the major occupation of the rural dwellers. Those living in rural areas were the population at risk and high risks groups were identified as those engaged in cleaning flooded areas after heavy rainfall. For the South American studies, the major risk factors reported were occupational hazards that increase contacts with animals, such as livestock farming and hunting among rural communities in the Amazon areas.

Table 9: Summary of the seven full text papers reviewed

Year	Title and design	Animal group	Geographical location	Summary	Reference
2008	A case control study to explore the risk factors for acquisition of leptospirosis in Surat city after flood	Rodent	Asia (India)	Study was an outbreak of leptospirosis due to flooding; those involved in cleaning and contacts with rodents were mostly affected.	(Bhardwaj et al., 2008)
2007	Cross sectional study; Control and prevention of rat fever leptospirosis outbreak in six villages of Raichur district Karnataka	Rodent	Asia (India)	Domestic rats confirmed as the main source of the pathogen; flooding also a risk factor.	(Masali et al., 2007)
2010	Cross sectional; Mild and severe clinical forms of urban leptospirosis; active outpatient based surveillance.	Rodent	El Salvador; South America	Sudden increase in urban rat infestation was associated with increase in incidence of leptospirosis.	(Calcagno et al., 2010)
2009	Cross sectional; Leptospirosis in the republic of Georgia.	Cattle	Asia (Georgia)	Study investigated leptospirosis as a cause of acute febrile illness. Primary animal contact the main source of infection.	(Clark et al., 2009)
2010	Case control study; Increasing trends of leptospirosis in northern India a clinicoepidemiological study.	Rodents and Livestock	Asia (India)	Infestations of dwellings with rats and occupational hazards due to contact with animals among farm workers were identified as the highest risk factors for <i>Leptospira spp.</i> infection.	(Sethi et al., 2010)
2008	Cross sectional study; Leptospirosis among patients with pyrexia of unknown origin in a hospital in Guwahati Assam	Livestock	Asia (India)	Leptospirosis as an occupational hazard among livestock and forest (wildlife) workers in the Guwahati Assam city in India.	(Kalita and Rahman, 2008)
2004	Case control; Leptospirosis in febrile men ingesting Agouti paca in south america	Wildlife Rodent (Agouti paca)	South America (Guyana)	Hunting and eating Agouti paca was identify as a high risk factor for leptospirosis among the Amazon hunters.	(Silverman et al., 2004)

1.4 DISCUSSION

This review describes patterns of reporting of zoonotic pathogens that cause human fever across malaria endemic countries. Overall, *Leptospira spp.* were the most reported among the zoonotic pathogens reviewed and were recorded mainly in Asia and Africa regions. In addition, *Leptospira spp.* were associated with more animal host species in comparison with other pathogens. It appears that *Leptospira* is a well-recognised cause of human febrile illness, which could have been affected by its epidemiological characteristics. Similarly, *Rickettsia spp.* were also frequently reported as a cause of human febrile illness. Leptospirosis and rickettsiosis affect high, middle and low-income countries, and are not exclusively diseases of poverty and marginalised communities with no political voice. It is also possible that momentum building and key research groups in Africa, Asia and Latin America are increasingly focusing attention on these diseases.

Findings of the full text reviewed papers that reported *Leptospira spp.* as cause of febrile illness, and animal associations, have elaborated on some of the reasons why the pathogen may have been frequently reported compared to others. The studies reviewed were all undertaken in low and middle income countries: India, Georgia, and Brazil, and in areas considered to be actively involved in agricultural farming with poor basic bio safety infrastructures leading to high risk of exposure in humans (Bharti et al., 2003; Masali et al., 2007; Silverman et al., 2004). For example, most of the studies were conducted in India, and involved cases of outbreak investigations (Table 9). The main risk factors identified were seasonal flooding from agricultural plants, farms and waste dumps to human settlements, especially in villages and peri-urban settlements, during heavy rain falls in monsoons (Bhardwaj et al., 2008; Kalita and Rahman, 2008; Sethi et al., 2010). In addition to the flooded rice farms, rodent population explosion was also observed after each flooding episode because displaced rats were struggling to find shelter after being deluged. These ecological and environmental factors could have significantly influenced the frequency of leptospirosis epidemics, which may have potentially increased interest, recognition and research activities of the pathogen in these settings. For instance, activities of the Global Leptospirosis Environmental Action Network (GLEAN) (Durski et al., 2014), an organization that was developed to improve global and local strategies on how to predict, detect, prevent, and intervene in leptospirosis outbreaks in order to improve prevention and control of leptospirosis in high-risk populations, would likely focus attention in these regions

where outbreaks are mostly reported. This may have probably led to less reporting of the disease in other highly endemic areas (such as sub-Saharan Africa) where outbreaks are not often reported.

A noticeable observation in this study was the high number of abstracts that reported Non-typhoid *Salmonella spp.* (NTS) as cause of human fever. The pathogen has been widely neglected in the past with more attention being given to typhoid (Bouzenoune et al., 2011; Thorns et al., 2000). It is difficult to differentiate the clinical manifestations of both pathogens in endemic regions, which may have resulted in misdiagnosis and under reporting (Thorns, 2000). However, the emergence of NTS as frequently reported cause of fever may suggest increasing recognition of the pathogen. This observation is consistent with several studies in sub-Saharan Africa that reported the bacteria as one of the most prevalent pathogen in bloodstream infections, and as an important cause of febrile illness especially, in immune comprised individuals (Reddy et al., 2010). The results also indicated that the pathogen was frequently reported in Africa as a cause of human fever, which is also in agreement with studies elsewhere that reported NTS disease was responsible for a larger disease burden than enteric fever, causing more than 100 000 deaths per annum (MacLennan, 2014). In addition, increasing recognition of NTS appears to coincide with malaria decline, thereby suggesting NTS cases that would have been misdiagnosed are properly being identified (Reddy et al., 2010).

While fever may also be a clinical presentation for Rabies virus and *Francisella spp.* infections in humans, they are not frequently reported as cause of fever. Therefore, these two pathogens may be an unlikely aetiology of fever in malaria endemic countries. Rabies virus, a well-known zoonotic pathogen, is a classical neurological disease in humans and with little or no fever observed especially at the early stages of the infection (Susilawathi et al., 2012). This may be one of the reasons why there were only three papers filtered for rabies. However, it is also possible that rabies is being misdiagnosed or under-reported due to the similarities with malaria in the neurological manifestation of the disease, as had been reported in Malawi (Mallewa et al., 2007). A similar case can be made for *Francisella spp.*; it is understood that the species of the pathogen that cause human disease is relatively uncommon in Africa and other tropical and sub-tropical malaria countries (US, CDC) that were covered in this review.

Leptospira spp. and *Rickettsia spp.* were the two pathogens that were mostly implicated in co-occurring infections in the sampled febrile population. The two pathogens were also the

most frequently reported, and constituted more than half of the incidents of concurrent reporting, which may suggest the potential for mixed infection in endemic areas. Another example of concurrent reporting was *Rickettsia spp.* and *Coxiella spp.* These pathogens, similar to *Leptospira spp.*, have a multiple host range, are widespread, and share risks factors for transmission (Blair et al., 2004; Zhang et al., 2011). High risk population, such as those in close contact with animals (e.g. animal handlers) (Schoonman and Swai, 2009), and those living in rural areas (Biggs et al., 2011), is similar for these pathogens. Even though *Rickettsia spp.* are largely transmitted by arthropod vectors, animals are still the main host of the pathogens and close contact likely to be important in transmission (Blair et al., 2004; Zhang et al., 2011).

Although all reviewed pathogens were reported in Asia and Africa, only a small proportion in Latin America and the Caribbean. It is possible that this reflects a genuine difference in prevalence or recognition across these regions. As explained earlier, major drivers of leptospirosis outbreaks are more prevalent in southern and southeast Asia (Bhardwaj et al., 2008), which may have increased the recognition and reporting of the pathogen. Similarly, the increase awareness of socioeconomic factors such as values attached on animals and communities living in close proximity with their animals (Akililu, 2008), may have also increase recognition and reporting of zoonotic pathogens in some regions such as sub-Saharan Africa. These factors could result in increased prioritization of leptospirosis research, and thus, the number of research publication from these regions. Another potential reason for relatively low reporting of studies in Latin America and the Caribbean could be due to improved livestock management practices in comparison with other malaria endemic regions.

There are 98 countries classified by World Health Organization (WHO) as malaria endemic (WHO, 2015b). Only 50 of these countries were represented by at least one of the studies reviewed. It is likely that the pathogens are also present in these other 48 countries because they are known to have a widespread geographical distribution. However, it is possible that the reviewed pathogens were not reported in these countries because they are probably being poorly recognised as cause of human febrile illness. Another reason could be that the search was biased by language since only abstracts published in English were considered and quality of journals because no local or national journals were considered.

The 10 zoonotic pathogens reviewed in this study are well recognised cause of zoonoses. However, only 44% of articles that were designed to consider risk factors investigated the

role of animals as risk factors for zoonotic disease transmission. This outcome is surprising, because the role of animals in the transmission mechanisms of zoonotic diseases is well recognised in the literature. Therefore, it would have been expected that a much larger proportion of studies that investigated risk factors of zoonotic pathogen transmission, would consider animal associations. The majority of the studies that investigated risk factors, while not considering animals, specifically addressed cultural, political, and socioeconomic demographic factors such as living in the rural areas, level of education and poverty, as predisposing factors for acquiring zoonotic infections. Even though these factors are important for zoonotic pathogen transmission and could be regarded as proxies for animal contacts, the integral roles of animals were not specifically described. It appears there is more emphasis on the clinical management of zoonotic disease at individual patient level than considering risk factors. While effective clinical management of human cases is important, preventive measures that address animal sources of infection and transmission routes are likely to be critical for protecting the disadvantaged communities, particularly, poor livestock-keepers who may have little access to effective health services (Halliday et al., 2015). Possible preventive measures could include livestock vaccination for diseases such as leptospirosis, Q fever, and brucellosis, and One Health approaches that integrate community healthcare workers and local livestock officers to create awareness about zoonotic diseases.

Non-typhoidal *Salmonella* was not identified with any animal species (including food-borne sources) and was mostly reported in Africa. There was no clear explanation for this observation, but in most endemic settings *Salmonella* infections are mainly considered human to human transmitted and may be the reason why studies rarely consider animals in the transmission of the pathogens (Feasey et al., 2012; Thorns, 2000). A review of community-acquired bloodstream infections in Africa identified that the high prevalence of non-typhoidal *Salmonella* among immune compromised individuals was mainly due to personal hygiene (Reddy et al., 2010).

The pattern of reporting of zoonotic pathogens as demonstrated by this review indicates that more publications were in life sciences and medical journals rather than veterinary journals. This could be expected since the abstracts include information on zoonotic pathogen in humans with fever. Perhaps, it may also reflect a growing awareness in the human health sector about the importance of zoonotic diseases even though animal related risk factors are not substantially described. It does provides an assurance that human health sector are increasingly recognising the importance of zoonoses.

In summary, zoonotic pathogens as cause of human febrile illness were not reported in all malaria endemic countries. They were reported mostly in specific regions of Asian and African continents. It is possible that areas where potentials for outbreaks exist are likely to be associated with increased research activities, recognition and reporting of the endemic zoonotic pathogens. However, despite the diagnosis of zoonotic pathogens as a cause of fever in the reviewed studies, the roles of animals in the transmission of these pathogens among other risk factors were often not considered and may not have been fully appreciated.

Leptospira spp. was confirmed as the pathogen most frequently reported as cause of human febrile illness in endemic settings. Furthermore, where animal hosts were reported in association with these studies, as seen in the case of *Leptospira spp.*, the full suite of potential animal reservoirs and/or source populations may not have been considered. The veterinary literature indicates that many animal species are important in the epidemiology of leptospirosis (Adler and de la Peña Moctezuma, 2010; Adler, 2001) and the role(s) played by other animal populations in the transmission of *Leptospira spp.* to people may be underestimated. This point is explored further in the next chapter.

CHAPTER TWO

Risk Factors for Bovine Leptospirosis in Northern Tanzania

2.1 INTRODUCTION

Leptospirosis is one of the most widespread livestock diseases in sub-Saharan Africa (Schoonman and Swai, 2009) and affects cattle worldwide (Adler and de la Peña Moctezuma, 2010). However, the burden of this disease has been greatly reduced in high-income countries through the implementation of comprehensive vaccination programmes and good bio-security measures (Ryan et al., 2012). This has not been the same in sub-Saharan Africa and most other developing countries where leptospirosis remains endemic in cattle and even widely neglected (Assenga et al., 2015). In leptospirosis endemic countries, the overall burden of disease is not fully appreciated by the affected farmers or policy makers. There is lack of knowledge and awareness about the disease impacts on livestock productivity and performance, and of the potential for zoonotic transmission to humans (Schoonman and Swai, 2009). In addition to awareness issues, many cattle farmers in livestock-keeping communities where bovine leptospirosis is endemic, may not be able to afford the cost of an effective vaccination programme against the disease without government subsidies (Ngbede et al., 2012a). These reasons may explain why, although livestock vaccines against some *Leptospira* serovars (such as *L. Pomona*, *Grippotyphosa*, *Canicola*, *Icterohaemorrhagiae*, and *Hardjo*) do exist and have been widely used to control leptospirosis in cattle in many developed countries, vaccination is rarely implemented in most of Sub-Saharan Africa and the disease remains highly endemic in the region (Allan et al., 2015; Assenga et al., 2015; Scolamacchia et al., 2010).

Bovine leptospirosis is caused by the genus *Leptospira*, which consists of a wide range of pathogenic and saprophytic spirochaetes. There are 6 saprophytic and 14 known pathogenic species of *Leptospira* and new species continue to emerge (Picardeau, 2013). There are more than 300 serovars of *Leptospira*. The term serovar refers to the basic unit of leptospires taxonomy, and a given serovar consists of isolates that share common serological properties of their lipopolysaccharide (LPS) (Bharti et al., 2003). The diversity of the *Leptospira* serovars is driven by the expression of the outer membrane carbohydrate, lipopolysaccharide (LPS), which differentiates the serovars of the same species (Picardeau, 2013). A serovar can belong to more than one species, for example serovar *Hardjo* includes *Leptospira* strains of

both *Leptospira interrogans* and *Leptospira borgpetersenii* (Levett, 2001). The 300 known serovars of *Leptospira* are clustered in to 24 serogroups world wide. Serogroup, for example *L. Icterohaemorrhagiae*, was previously used to classify *Leptospira spp.* Serogroups do not have a formal taxonomical classification, but represent serovars that are antigenically related and can be determined by microscopic agglutination test (MAT) (Budihal, 2014). There is also cross reaction between these serovars of *Leptospira*, which makes the use of serology to determine prevalence of each serovar quite complex (Adler and de la Peña Moctezuma, 2010).

2.1.1 Transmission of bovine leptospirosis in endemic settings

Transmission of leptospirosis involves three broad routes, environmental transmission (which includes direct or indirect contact), vertical or maternal transmission, and sexual transmission (Adler and de la Peña Moctezuma, 2010). The main mode of transmission in cattle is through direct or indirect contact with infective materials (such as urine or milk where large quantity of the pathogen is excreted), and this route of transmission may depend on farm hygiene and bio-safety measures. Urine contamination of feed and drinking water has been identified as a major source of herd infection in tropical Africa (Schoonman and Swai, 2010). At farms with poor biosecurity practices, especially where quarantine facilities are not available, sick or apparently healthy animals that are shedding leptospires can contaminate feeding and drinking troughs, which can encourage the spread of infection (Schoonman and Swai, 2010). The isolation of *Leptospira spp.* in milk (Thiermann, 1981), indicates that lactating cows can transmit infection to suckling calves, and infection can also be potentially spread by environmental contamination through movement of milk and other dairy products in the farms. Other modes of transmission are possible in cattle. The detection of pathogenic *Leptospira spp.* in vaginal mucous and semen strongly supports evidence of sexual transmission (Adler, 2014).

In endemic areas where routine vaccination and surveillance is not practiced, these modes of transmission can be critical because they are difficult to control even with good farm practice. Several other livestock species, rodents and wildlife have been identified as hosts of infection, and considered to play significant roles in the transmission of the disease in cattle (Lau et al., 2012; Schoonman and Swai, 2010). This wide range of hosts, in addition to multiple

transmission routes, suggests that prevention of infection in cattle without vaccines will be quite challenging especially in extensively managed systems.

There are still many gaps in our understanding of the factors that drive leptospirosis transmission patterns in livestock populations in sub-Saharan Africa. The disease has a complex epidemiology, and identifying the effect of these factors will be important in designing strategies for the prevention and control in animal populations. Factors such as herd size and livestock production systems, which have been previously suggested to have effect on the distribution of endemic infectious diseases such as bovine tuberculosis (Cleaveland et al., 2005), foot-and-mouth disease (FMD) (Lembo et al., 2012), brucellosis (McDermott and Arimi, 2002), have also been associated with leptospirosis (Assenga et al., 2015). However, the transmission dynamics in cattle populations and factors that affect the endemicity of the pathogens and the patterns of local spread for different serovars in sub-Saharan Africa is not clear. It has been suggested that environmental factors that support the survival of the pathogens, animal husbandry systems that allow unrestricted movement of animals across regions, lack of vaccination of livestock population against the disease, and poor bio-safety and farm hygiene that encourages interaction between livestock and rodents, may all increase the prevalence of the disease (Lau et al., 2012; Schoonman and Swai, 2009; Scolamacchia et al., 2010).

2.1.2 Serovars, host range and prevalence of leptospirosis

The domestic animals cattle, sheep, goats, pigs, horses, dogs and rodents have all been considered maintenance hosts of *Leptospira spp.* (Adler and de la Peña Moctezuma, 2010). However, serovars and host classification of *Leptospira spp.* largely depends on region and specific ecological and environmental features that encourage host abundance. For example, host-adapted serovars are considered to cause chronic or subclinical infections, while non-host adopted serovars are usually involved in acute clinical infection (Pinna et al., 2014; Yan et al., 2010). Detail of the host range of different serovars is presented in Table 1.

Table 1: Host range of different *Leptospira* serovars as reported by several studies in different regions

Animal group	Major host or common types/serovars	Other serovars
Cattle	Hardjo (Assenga et al., 2015; Schoonman and Swai, 2010)	Icterohaemorrhagiae, Pomona (Aisser et al., 2013); Grippotyphosa, Hebdomadis, Australis (Niang et al., 1994; Schoonman and Swai, 2010)
Sheep	Autumnalis, Grippotyphosa (Niang et al., 1994)	Pomona, Hardjo (Niang et al., 1994)
Goat	Pomona, Hardjo (Niang et al., 1994)	Hardjo, Autumnalis, Grippotyphosa (Niang et al., 1994)
Pigs	Pomona, Bratislava, Tarassov (Bolin and Assells, 1992; Bolin et al., 1991)	Canicola, Hardjo, Grippotyphosa, Icterohaemorrhagiae (Bolin and Assells, 1992; Bolin et al., 1991).
Horse	Bratislava, Icterohaemorrhagiae (Lees and Gale, 1994)	Bratislava and Icterohaemorrhagiae, Copenhageni, Australis (Lees and Gale, 1994)
Dogs	Canicola, Mini (Desvars et al., 2012)	Pomona, Grippotyphosa, Australis, Hardjo (Desvars et al., 2012)
Rodents	Copenhageni (Lees and Gale, 1994)	Almost all serovars have been identified (Assenga et al., 2015; Lees and Gale, 1994)
Humans	None specific	Mostly: Mini, Australis, Hebdomadis, Bratislava and Icterohaemorrhagiae (de Vries et al., 2014; Desvars et al., 2012)

There is limited information about the prevalence of the pathogen in domestic animals species in Sub-Saharan Africa (Allan et al., 2015; de Vries et al., 2014). In cattle for instance, there are several species and subtypes of the pathogen and the variations depend on the

region where the serovar was isolated (Allan et al., 2015). The serovar Hardjo is considered the most dominant type and has the highest prevalence in East Africa (Assenga et al., 2015). Other serovars such as Pomona, Icterohaemorrhagiae, Canicola, and Grippotyphosa, have also been identified in cattle in the same region, but with much lower prevalence (Assenga et al., 2015; Schoonman and Swai, 2010). One of the recent cross-sectional studies conducted in the Katavi region, which is predominantly agropastoral cattle farming, in Tanzania reported an overall (all serovars) seroprevalence of 30.4% in cattle using Microscopic Agglutination Test (MAT) technique (Assenga et al., 2015). The serovar Hardjo was identified as the major circulating type with serovar-specific seroprevalence of 17.6% of all cattle tested (Assenga et al., 2015). Another sero-survey undertaken in small farm holders and extensively managed cattle in the Tanga region reported seroprevalence of 30.3% for all serovars (Bataviae, Tarassovi, Hardjo and Pomona), and the serovar Hardjo was also the dominant circulating type with a seroprevalence of 15% of all cattle tested (Schoonman and Swai, 2010).

In Western Africa, cross sectional studies and abattoir surveys that screened cattle using enzyme-linked immunosorbent assay (ELISA) as the laboratory diagnostic methods, indicated a variation in the prevalence of bovine leptospirosis, and also identified serovar Hardjo as the main circulating type. Studies conducted in northern Nigeria reported a prevalence of 3.5% in an abattoir survey (Ngbede et al., 2012a), and two cross sectional studies conducted at different periods in the same region reported a prevalence of 8.4% (Ngbede et al., 2013) and 11% (Ngbede et al., 2012b). A serosurvey of communal cattle herds conducted in Mali using MAT, reported an overall prevalence of 44.8%, with Hardjo specific prevalence of 13%, Pomona(9.2%), Grippotyphosa (6.4%), Hebdomadis (6.2%), and Pyrogenes(5.2%) (Niang et al., 1994).

Very few studies on bovine leptospirosis have been reported in Central Africa (de Vries et al., 2014). Analysis of 1377 samples from 146 herds that were sampled in a cross sectional study in the Adamawa province of Cameroon, estimated prevalence of 30.4% for *Leptospira* Hardjo by ELISA after adjusting for diagnostic test performance and study design (Scolamacchia et al., 2010).

The overall seroprevalence and the dominant circulating types of bovine *Leptospira* serovars appear different in endemic regions in Asia compared to sub-Saharan Africa. Studies conducted in Turkey reported seroprevalence of 14% by MAT for all identified serovars, and the serovar Grippotyphosa was identified as the major type in 57% of the positive sera

samples (Aslantaş and Özdemir, 2005). In another region in Asia, *L. interrogans* and *L. borgpetersenii* were identified as the most prevalent types, and primary cause of bovine leptospirosis (Kocabiyik and Cetin, 2004). However, findings in Iraq are similar to those reported in West Africa. A cross sectional study on naïve adult cattle using a commercial ELISA kit reported a prevalence of 6.5% for serovar Hardjo and 1% Pomona (Aisser et al., 2013). These further demonstrate *Leptospira* serovar Hardjo as the predominant circulating type. Overall, the pattern and importance of different circulating types depends on the region and period the study was conducted.

2.1.3 Review of some risk factors for bovine leptospirosis

Livestock production systems

The nature of animal husbandry systems in sub-Saharan Africa could be a driving force in determining the prevalence of leptospirosis (Schoonman and Swai, 2009). Pastoral systems for example, typically involve large herds, with unrestricted movements and opportunities for contacts with wildlife. There are several possibilities for sharing water sources and grazing areas potentially exposing large numbers of cattle to infection. Pastoral management systems also enhance possible mixing of cattle and wildlife that may be acting as reservoirs of infection (Gangadhar et al., 2000). Extensive mix-species management systems that allow co-grazing of cattle with other ruminants such as sheep and goats could also increase the transmission opportunities to cattle.

Genetic disposition

Many questions remain as to whether certain breeds are more predisposed to infection with *Leptospira spp.* than others, and whether there are breed adapted and non-breed adapted serovars (Picardeau, 2013). There is not enough evidence in the literature at this point in time to support or refute these suggestions. However, a recent study undertaken in India observed no difference between breeds in terms of the probability of animal seropositive status (Patel et al., 2014), while a study conducted in West Africa, Nigeria, reported a significant difference between the seropositive status of an indigenous breed of cattle when compared to cross and exotic breeds (Ngbede et al., 2013).

Age

Age has been identified as a major factor that affects the seroprevalence of *Leptospira spp.* antibodies in cattle. Evidence from studies conducted in sub-Saharan Africa (Ngbede et al., 2012a; Schoonman and Swai, 2010), and Asia (Kocabiyik and Cetin, 2004), reported a significant increase in the seroprevalence of leptospirosis with age of cattle where antibody titre peaked at the older animal age groups. The likelihood of repeated exposure as animals get older was described as the main reason for age differences in cattle (Schoonman and Swai, 2010).

Sex

Varying relationships between cattle *Leptospira* seropositivity and sex are described in the literature. Epidemiological studies undertaken in Turkey (Aslantaş and Özdemir, 2005), India (Patel et al., 2014) and West Africa (Ngbede et al., 2012a), reported no significant association between the sex of cattle and exposure to leptospirosis, while a study in Mali (West Africa) reported that cows are significantly more likely to be seropositive compared to bulls (Niang et al., 1994). Despite the contrasting views about the effect of sex on cattle seropositivity, factors that relate to production and performance problems such as abortion and reduced milk production have been well documented as a major outcome of the bovine leptospirosis, which suggests a higher economic impact of infection in female animals.

Ecological features

The environment is considered to play a major role in maintaining the abundance of leptospires in circulation. Soil type, elevated ground levels, proximity to rice fields, have all been shown to help improve the survival rate of the *Leptospira spp.* in the environment (Lau et al., 2012; Schoonman and Swai, 2010). In addition, other environmental and climatic factors such as heavy rainfall and flooding, high temperatures, poor sanitation and waste disposal, that may increase pathogen abundance, have been associated with high incidence of leptospirosis (Levett, 2001; Maskey et al., 2006). However, the importance of the environment may also vary for different *Leptospira spp.* For example, it has been reported that *L. Pomona* appears to be more prevalent in areas of low rainfall compared to *Leptospira* serovar Hardjo with high prevalence in high rainfall settings (Elder and Ward, 1978).

2.1.4 Challenges in controlling bovine leptospirosis in endemic areas

Various economic and political instability problems in sub-Saharan Africa, in addition to poor veterinary infrastructure, have contributed to difficulties in controlling most endemic livestock diseases. Disease control programmes are inevitably prioritized because of limited resources, leading to several endemic diseases being neglected. Leptospirosis is among the diseases being considered by World Health Organization (WHO) as neglected in most low and medium income countries where they are endemic. Currently, research and surveillance activities into leptospirosis in these traditional endemic settings are not properly coordinated to effectively control the disease, leading to very scarce data on bovine leptospirosis (Allan et al., 2015; Halliday et al., 2015).

Difficulties in detecting and diagnosing leptospirosis in poor countries have further limited the disease surveillance efforts. Bovine leptospirosis may be asymptomatic especially if infected with a host adaptive serovar (Adler, 2014), and can easily be confused with other endemic animal diseases such as brucellosis and coxiellosis in acute stages of the infection (Allan et al., 2015; Halliday et al., 2015). This means adequate laboratory diagnosis would be required for disease confirmation and to allow administration of appropriate treatment. Understanding the risk factors for infection and transmission dynamics have also been very challenging in endemic areas. Environmental persistence of the pathogen, poor biosecurity and farm hygiene have also complicated control options particularly in tropical areas where climatic conditions favour prolonged bacteria survival (Lau et al., 2012). In addition, heavy rainfall and flooding have all been identified as risk factors for human infection (Levett, 2001), and can also enhance transmission in livestock.

Livestock vaccines are currently available for some serovars of leptospirosis, but they are rarely used in sub-Saharan Africa, particularly in traditional and smallholder sectors (Assenga et al., 2015; Ezeh et al., 1990). The lack of awareness of the disease impact among livestock-keeping communities, the cost of vaccine and treatment, unavailability and access of the vaccines in some areas, have all been reported as reasons for not adopting available control measures (Allan et al., 2015; Halliday et al., 2015).

2.1.5 Clinical signs of bovine leptospirosis

In sub-Saharan Africa, clinical signs of a disease are an important aspect of primary diagnostic criteria used by veterinarians because of the challenges in making a full laboratory diagnosis. However, leptospirosis can present with a wide range of clinical signs, which may vary with species, age and sex. The clinical signs seen can also depend on the level of herd immunity and on the infecting serovars (Adler, 2014; Levett, 2001). Chronic infection with host adapted serovars are normally mild with less obvious signs, while there could be a more severe infections in acute stages or with non-host adapted serovars (Adler, 2014; Levett, 2001). For example, *Leptospira* serovar Hardjo is considered to be host adapted in cattle and chronic infection with this serovar has been associated with mild illness (Adler, 2014). In contrast, acute phases of infection or exposure to non-host adapted serovars like Canicola or Pomona results in more severe illness (Evangelista and Coburn, 2010; OIE, 2014). The major clinical signs may depend on age of cattle, ranging from mild infection in adult animals to a severe life threatening disease in young animals (Bharti et al., 2003; Evangelista and Coburn, 2010; OIE, 2014). Calves may show sudden onset of febrile illness, jaundice, and laboured breathing and sometimes death in 3-5 days may be observed (Adler, 2001). The observed signs in adult cattle vary. In acute infection, abortions of up to 30% have been recorded and occur at the fourth- seven month of gestation (OIE, 2014). Stillbirth and infertility problems have all been reported (Bharti et al., 2003). Milk drop syndrome can also occur in the early stages of the infection with milk becoming thick and yellowish (OIE, 2014; Sethi et al., 2010). The majority of animals will normally recover from the disease, however, an overall case-fatality rate of 5% has been recorded and there could be further complications if there is co-infection with other pathogens (Ellis et al., 1985).

2.1.6 Serological response after exposure

The incubation period of the pathogen in cattle regardless of the source of exposure, varies from 2-30 days (Adler, 2014; Mazzonelli, 1984). There are three stages of the leptospirosis infection cycle in cattle, similar to other species such as humans (Levett, 2001). The first stage is the acute stage (week 1) of the disease, where the leptospires start circulating in the blood (leptospiemia) for about one week (Mazzonelli, 1984). The host immune systems

detect the presence of leptospire antigens and react by producing antibodies (Adler, 2001). At this early stage of the disease however, the antibody titre is low and may not be detected by serological tests (Spickler et al., 2013). The major clinical signs observed in this first stage of infection are mainly fever and there may also be abdominal pain, loss of appetite, lethargy and muscle tenderness (Evangelista and Coburn, 2010; OIE, 2014). The second stage of infection in cattle is the convalescent stage (week 2 – and 3). At this stage, the antibody titre rises sharply. The initial serological detection of antibodies is usually from the 10th-14th day post exposure and titres often peaked from the 3rd week (precisely around the 15-18th day post exposure) (Musso and La Scola, 2013). High antibody titres may persist for 3-6 weeks or may wane sharply depending on the immune status of the animal (Spickler et al., 2013). The third stage of infection is the chronic stage, which may start from the 4th week post exposure onwards and may sometimes overlap with the convalescent stage. The antibodies start to wane gradually at this stage as the pathogens are cleared from the blood and tissues (Pedersen et al., 2015). The leptospire that survive the attack from host immune systems, colonize host tissues, especially the convoluted tubules in the kidneys, from where their infective stages are being shed in urine for weeks, months or even years and such hosts could potentially become carrier (Reinhardard, 1951). Animals in the carrier stage may not produce detectable antibody titre against the pathogen or show clinical signs of infection. However, the animals may still shed the pathogens (Reinhardard, 1951) in the environment, and therefore pose a great risk for transmission to other animals and humans (Pedersen et al., 2015).

2.1.7 Laboratory diagnosis of bovine leptospirosis

Indirect detection

Indirect diagnostic methods such as serological test using ELISA or Dipstick are often used in most national veterinary diagnostic centres in Africa (de Vries et al., 2014; Musso and La Scola, 2013). However, routine diagnosis of bovine leptospirosis in the field is not a common practice. ELISA is often regarded as a cheaper, affordable, and less complicated option compared to other indirect methods such as MAT (Budihal, 2014; de Vries et al., 2014). Several ELISA are available commercially and are particularly useful as indirect evidence to identify the immunological response of cattle that have been exposed to *Leptospira spp* usually at the 10-14 day post exposure (Musso and La Scola, 2013). IgM detecting ELISAs

are more sensitive in the early phase of infection, while IgG specific ELISA will be more effective in the later stages (Goris et al., 2012; Musso and La Scola, 2013). In most developing countries where diagnostic capacity for veterinary services is limited, ELISA is frequently used as the only diagnostic tool to establish infection (Musso and La Scola, 2013). Positive serology results by ELISAs suggest a probable case of leptospirosis, but cannot confirm the presence (or absence) of the disease, especially in adult animals that may have high background exposure (Budihal, 2014).

The Microscopic agglutination test (MAT) is the gold standard serological test for leptospirosis (Goris and Hartskeerl, 2014), and the most commonly used reference diagnostic test for bovine leptospirosis (Goris and Hartskeerl, 2014). The MAT is a serogroup specific test, which indicates the circulating serovar (or serogroups). The MAT is not usually sensitive and reliable at the acute phase of infection and may not be appropriate to detect early stages of infection (Cumberland et al., 1999). Paired sera are often required to establish seroconversion (fourfold increase in MAT titre between acute and convalescent phase serum samples) (Cumberland et al., 1999). This remains a huge challenge in certain veterinary practice where for example animals may not be available for a second sample collection because they could have been sold or even slaughtered. However, a diagnostic MAT titre of >1:400 in a single serum sample in association with specific clinical signs, may be considered a confirmatory diagnosis (Goris et al., 2012). In the acute phase of infection, cross-reaction among the serovars in the MAT test panel makes the interpretation of the serovar or serogroup specificity of antibodies very difficult.

Direct detection

Leptospira spp. can be detected directly through isolation of the pathogenic species from infectious material in the laboratory (culture), dark field microscopy (DFM), detection in clinical samples by histology or immunostaining techniques and through identification of the pathogen genetic materials (DNA) by Polymerase chain reaction (PCR) (de Vries et al., 2014; Musso and La Scola, 2013). In the acute leptospiremic stages (usually within the first 10 days) of infection, leptospires can be cultured from blood and cerebrospinal fluid of infected individuals (Levett, 2001). However, the culture procedure is cumbersome and time consuming because it takes weeks for the pathogen to grow on culture and may not be ideal for veterinary use (Hartskeerl et al., 2011). Dark field microscopy can be used to visualize the pathogen in the leptospiremic phase, but lacks specificity and sensitivity and a large number

(10^4 /ml) of leptospires are required in each field to be visualized (Budihal, 2014). The molecular diagnostic test PCR is increasingly being used as a confirmatory test for bovine leptospirosis in developed countries, but not common in low resource countries because of the required techniques, expertise and costs (Budihal, 2014; Hartskeerl et al., 2011; Musso and La Scola, 2013). It is ideal to detect leptospires in the acute leptospiremic stage of infection (within the first week) when the pathogen freely circulates in the blood and cerebrospinal fluid (CSF) (Levett, 2001; Musso and La Scola, 2013). In the later stages of the infection where the leptospires have been cleared from the blood and CSF and colonized in the kidneys, PCR may be insensitive to identify the pathogens (Pedersen et al., 2015). However, kidney samples from the abattoir will provide a very useful source for pathogen detection using PCR even when clinical signs are not present at time of slaughter.

2.1.8 The impacts of bovine leptospirosis

The livestock industry makes a very significant contribution on the gross domestic product (GDP) of most sub-Saharan countries. In countries like Tanzania, which has the second largest livestock population in Africa, it contributes about 13% of the GDP (Ministry of Livestock and Fisheries Development, 2010). Livestock are kept as source of food and livelihood for most households and could be their only source of income (Aklilu, 2008). Diseases such as leptospirosis, which affect the productivity and performance of animals, will have huge impacts on food security and livelihoods of many households (Onono et al., 2013; Torgerson et al., 2015). Production related losses due to leptospirosis can potentially provoke hunger, poverty and suffering among livestock-keeping communities. There could also be additional costs of veterinary services, vaccines and treatment of affected animals. Awareness as regards to the disease impact is often poor and affected farmers can hardly recognise leptospirosis as reason for poor performance of their animals and the true impact of the disease is completely under evaluated (Taylor et al., 2015).

Leptospirosis is also a zoonotic disease with huge burden on human health (Allan et al., 2015). There are currently no available data on the overall economic significance of the disease in sub-Saharan Africa. The disease is distributed worldwide and human infections are very common (Matthias et al., 2008). In humans, the clinical signs and symptoms are generally non-specific and often confused with other diseases of similar signs (Biggs et al.,

2011; Crump et al., 2013; Halliday et al., 2015). However, the disease is associated with febrile illness, jaundice, haemorrhagic renal failure, neurological and pulmonary symptoms, which may have significant impact on human health (Lau et al., 2012).

Human infection occurs when there is direct or indirect contact with infective leptospires from infected animals. Prevalence depends on the location, and characteristics of the population such as a previous history of animal contact (Allan et al., 2015; Evangelista and Coburn, 2010; Matthias et al., 2008; Talpada et al., 2003). High risk groups that have been identified are those in close contact with animals such as slaughterhouse workers (including meat inspectors), dairy farm workers and other animal handlers (including veterinarians) (Brown et al., 2010; Schoonman and Swai, 2009). In addition, swimming in infected pool after heavy rain fall or walking with bare feet have also been identified as risk factors for infection (Bhardwaj et al., 2008).

The human burden of leptospirosis can be quantified in terms of standardised measures of disability-adjusted life years (DALYs) (WHO, 2015c). DALYs are used to measures the cost of time lived with a disability and the time lost due to premature death and are a widely used parameter to compare and assess the burden of a disease (Carabin et al., 2005). In addition, the Quality Adjusted Life Year (QALY) parameter is used to quantify health benefits in terms of the quantity and the quality of life of an individual (Torrance and Feeny, 1989).

Combining DALY and QALY parameters can be a very useful tool to determine the cost and benefits of government interventions towards the control of a zoonotic disease such as leptospirosis. However, there are current limitations in using these tools to assess the overall burden of leptospirosis and other zoonotic diseases because of their dual impacts on humans and animals (Grace et al., 2012). The human morbidity and mortality data on leptospirosis indicate that globally, approximately 2.9 million DALYs are lost per annum as a result of 1.03 million human cases, which cause an estimated 59,000 human deaths per year (WHO, 2015c). In these studies, the east sub-Saharan African region was identified among the regions of the world with the highest disease burden (Costa et al., 2015; Torgerson et al., 2015). However, the relative contribution of cattle-transmitted leptospirosis to the human disease burden is currently unknown and information on the true impact on livelihoods due to livestock related losses is not available.

2.1.9 Aims and rationale of the study

This study aimed to evaluate how livestock management related factors, such as animal husbandry practices, age, breed and sex, affect the seroprevalence of leptospirosis in cattle in Tanzania and the implications for human transmission. Determining the prevalence of exposure to *Leptospira* serovar Hardjo in the animal population will provide valuable data to inform our understanding of infection risks in different cattle husbandry systems, and can inform the design of potential control strategies. The specific aims are:

1. To determine the effect of animal husbandry systems, age, breed, and sex on the serostatus of Tanzanian cattle.
2. To describe the difference in seroprevalence between farm cattle and those slaughtered in the abattoir.

2.2 METHODOLOGY

2.2.1 Materials and methods

The samples and linked data used for this work were collected as part of a previous study, the Bacterial Zoonoses project. Section: 2.2.2 'Samples and data available from bacterial zoonoses study' describes the collection of the field data used for this study. This section has been included to provide a clear description of the sample collection and linked data available through the Bacterial Zoonoses project. All other sections represent the original work performed as part of this MSc.

2.2.2 Samples and data available from Bacterial Zoonoses study

The cattle serum samples used in this study were collected through two linked surveys conducted in northern Tanzania. A total of 96 samples were collected as part of an abattoir-based cross-sectional survey in which samples were collected from individual cattle processed at one of two cattle abattoirs in Moshi. Venous blood samples were collected from animals held in lairage before slaughter. In addition to blood samples, data was collected on the region of animal origin, age, breed and sex of all blood sampled animals. These data were collected between December 2013 and August 2014. Sampled cattle originated from Manyara, Singida, Arusha, and Dodoma regions. All the animals that were surveyed were adult and indigenous breed.

The second population of samples were collected through a cross-sectional study conducted in northern Tanzania. This was a household-based survey of livestock owning households stratified across peri-urban, agro-pastoral and pastoral settings in northern Tanzania. The survey was based in Moshi and included data collection from districts across the regions of Arusha and Kilimanjaro.

The selection of units for inclusion was performed using a multi-stage random selection technique as follows:

(a) Identification of districts within Kilimanjaro and Arusha regions

The 7 Kilimanjaro and 5 districts to the east of Arusha region were considered for selection. The only exclusion was Karatu and Ngorongoro districts, which were excluded based on

distance from Moshi and logistic constraints. The project team visited each of the 12 district authorities after sending out introductory letters about the project, to obtain basic information about the administrative structures within each district. Districts without initial response were followed-up for up to 2 more times and excluded if no response (Table 2).

Table 2: List of districts included in the study sampling frame

Region	District
Kilimanjaro	Rombo
	Mwanga
	Moshi District
	Moshi Municipal
	Hai
Arusha	Monduli
	Meru
	Arusha City
	Arusha District
	Longido

NB: The districts of Same and Siha within Kilimanjaro region were excluded due to no response after several follow up attempts.

(b) Identification and Characterization of Potential Study Wards

For each district that responded to the initial visits, a list of all wards within the district was obtained. The 2002 census was used as the basis of this list and this was updated in communication with district level officials. Detail of new wards that have emerged post 2002 was identified from the district administrators. All wards listed in the 2002 census were classified as Rural/Urban/Mixed based on the Tanzanian census enumeration areas. Data about the presence of significant pastoralist populations in each ward were obtained from livestock officers and veterinary administrators. If information about the presence of pastoralists was not available the ward was classified as not applicable (NA).

c) Setting Classifications

Wards were classified into one of the three setting classifications used in the Bacterial Zoonoses study: Pastoral, Agro-Pastoral and Peri-Urban. This classification was made using

information on the 2002 Census ward classifications on the data on the presence of significant pastoralist population as detailed below. Wards not meeting any of these criteria were excluded from the study.

Pastoral: This includes Wards in Longido and Moduli districts that are classified as Rural based on 2002 census ward classifications and are described as containing significant pastoralist populations by district level authorities.

Agro-Pastoral: This consists of Wards in Kilimanjaro region (Rombo, Moshi Rural, Moshi Urban, Hai and Mwanga districts) that are classified as Rural based on 2002 census ward classifications.

Peri-Urban: Includes Wards in Kilimanjaro region Rombo, Moshi Rural, Moshi Urban, Hai and Mwanga districts that are classified as Urban wards based on 2002 census ward classifications.

Selection of study wards

Nineteen wards were randomly selected for inclusion in the study. This included five wards from the pastoral setting, seven from the agropastoral and seven from the peri-urban. Sampling was performed with replacement so that an individual ward could be selected more than once but this did not occur. The wards were visited in a sequence that cycled through the settings. Within each setting, wards were visited in the order selected.

Village and Sub-village Selection within Study Wards

In each selected study ward, the team communicated with ward executive officers to confirm the full list of villages within the ward (using lists from 2002 census as starting point but including updated information provided by ward executive officer) and randomly select one of these villages. In pastoral and agropastoral areas villages consisted of several sub-villages and in these cases the team communicated with village executive officers to confirm the full list of kitongoji (sub-villages) and randomly selected one of these kitongoji. In peri-urban areas the unit equivalent to the village is known as an Mtaa (or street).

Selection of households within villages and sub-villages

The study team visited the selected Mtaa or Kitongoji to organize a rapid survey of household livestock ownership. This was organized by arranging for all of the Balozi (local leaders and

local administrative units) within the selected Kitongoji or Mtaa to gather data on the numbers of cattle, sheep and goats present at each household within their Balozi. Balozi were randomly selected and then individual livestock-owning households (households with at least one head of cattle, sheep, or goat) were randomly selected and approached for enrolment in the study.

Livestock sampling

For each enrolled household up to 15 cattle were sampled. Details of the sampling of sheep and goats are provided in Chapter Three. At households with >15 cattle, 15 individuals were selected and sampled. Selection of individuals was essentially opportunistic but adult females were prioritized. At households with ≤ 15 cattle all cattle were sampled. In addition to venous blood samples, data on the age, sex and breed of animals were collected. The ages of the animals sampled at households were determined using the standard dentition criteria based on the number of pairs of permanent teeth and the samples consist of animals of:

Temporary teeth only (1_Temp) - <12 months

2 permanent teeth eruption (2_2T) - 12-24 months

4 permanent teeth eruption (3_4T – 24) – 36 months

6 permanent teeth eruption (4_6T – 36) – 48 months

Full permanent teeth (5_Full) - 48-60 months

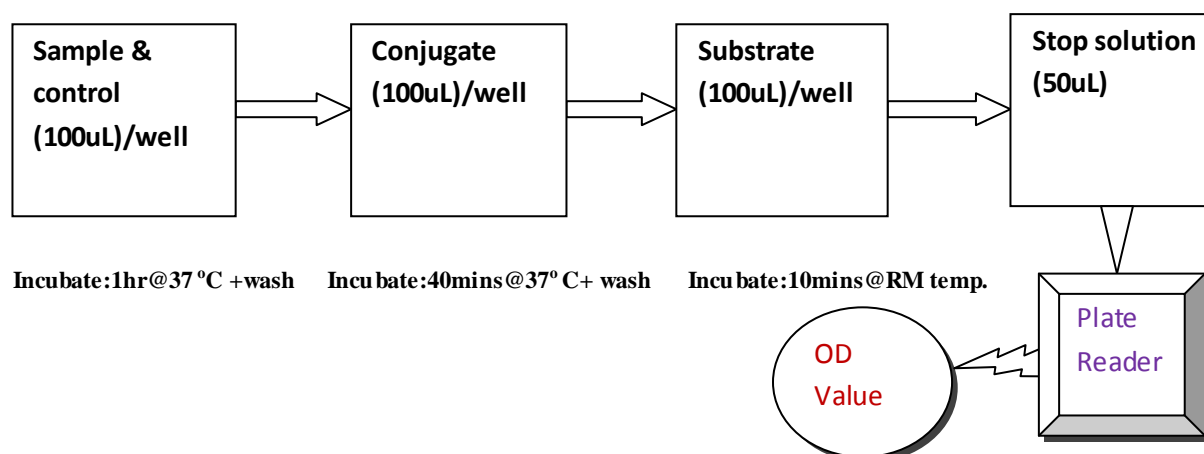
Full worn permanent teeth (6_Fullworn) – ≥ 5 years old

The breed of each animal was classified as one of the following options: indigenous, exotic or cross-breed. Information on the vaccination history of the animals was obtained at the time of sampling and no vaccination against leptospirosis was reported from any of the households visited through the cross-sectional study. These data were collected between July 2013 and November 2014.

All blood samples were collected into 10mL red-top vacutainers for serum separation. Whole blood was centrifuged to separate serum on the day of collection and serum samples were then refrigerated for up to 24 hours before transfer to storage in -80°C. Serum samples were heat treated in Tanzania to inactivate any potential harmful agents, shipped to the laboratory at the University of Glasgow on dry ice and upon arrival to the laboratory were immediately transferred and stored in -80°C freezers.

2.2.3 Serum sample handling

Figure 2: Protocol flow chart of the assay procedure



2.2.4 Serological testing

Test sample and control preparation: On each test day, the samples to be tested were thawed at room temperature. Test samples and controls were tested in duplicate, and for each test plate a total of 46 samples were tested (See Figure 1 for plate layout).

ELISA test kits: Each ELISA test plate was pre-coated with serovar Hardjo specific antigen, which binds with the serovar Hardjo specific antibodies in the test sera. The peroxidase antibody (conjugate) added to the reaction detected the antibodies that have been bound to the antigen and formed a matrix. Further addition of the ELISA substrate tetramethylbenzidine (TMB) enabled the amount of antibody present in each test well to be quantified. The optical density was then measured using a micro-plate reader at a wavelength of 450nm.

2.2.5 Test procedure

The test was carried out following the precise recommendations of the manufacturer (summarised in Figure 2), observing good laboratory practice and following similar test procedure as reported previously for similar studies (Ngbede et al., 2012a; Scolamacchia et al., 2010).

Dilution preparation for required working volume: The required volume for each test serum and control per reaction was 200µL (ie. 100µL for each test well which was done in

duplicate), however, 250 μL was prepared as a working volume to allow easy handling of the samples. To prepare a required dilution of 1:50, 5 μL of test serum and control were added to 245 μL of sample diluents. The wash buffer was supplied as 20X concentration and diluted to working volume (20ml of concentrated wash buffer was added to 380ml of distilled water) prior to testing. The peroxidase conjugate antibody was always freshly prepared for each test reaction. A test volume of 11000 μL was used for each test plate. This was prepared by adding 11 μL conjugate to 10989 μL diluted wash buffer. Substrate and stop solution were ready for direct use.

Test reaction: A multichannel pipette was used to transfer 100 μL of test samples and controls from dummy plate wells into ELISA test plate. Each test plate was covered with sealer and incubated for one hour at 37°C without shaking. The wells were washed using an automatic plate washer. 100 μL of diluted conjugate was then added to wells, covered with plate sealer and incubated at 37°C for 40 minutes without shaking. The wash step was repeated as above. Then 100 μL of substrate was added to each test well, covered with plate sealer and incubated for 10 minutes at room temperature in a dark room. Finally, 50 μL of stop solution was added to each well in the same order as the substrate was added. The micro plate reader was programmed for a quick shake for 10 seconds and the plate was read at a single wave length of 450nm.

Test interpretation: The micro plate reader measures the optical density for each well. Since the tests were done in duplicate, the average OD for the each sample and controls were determined. For each sample, the results were expressed as a ratio of the difference of the OD of the samples and the negative control to the difference of the OD of the positive control to the negative control.

$$\text{Ratio} = \frac{\text{Mean Sample OD} - \text{Mean Negative control OD}}{\text{Mean Positive control OD} - \text{Mean Negative control OD}}$$

Details of the test interpretation are shown in Table 3. The result range of 0.05 - 0.12 was regarded inconclusive and the assay manufacturer recommendation that animals should be retested. However, samples classified as inconclusive based on the kit recommendations were not retested and were classified as negative for this study.

Table 3: Sample ratio values and interpretation (Kavanagh et al., 2002)

Ratio value	Result	Interpretation and field relevance
Sera ≤ 0.05	Negative	Naïve and/or have not been vaccinated
$0.05 < \text{sera} \leq 0.12$	Inconclusive	Retest in triplicate and average OD. If still inconclusive, animal can be considered negative.
sera > 0.12	Positive	Have been exposed to infection/ have been vaccinated.

Quality control: Test plate was regarded invalid if the OD for the negative serum controls was >0.25 using the single wavelength of 450nm. There was no stated minimum value for the positive control based on the manufacturer's guidelines, but test will not be valid if the OD for positive control was less than negative control.

2.2.6 GIS maps of study sites

All the maps presented in this thesis were developed using the QGIS software (QGIS Development Team, 2016). Shape files for the study area (Arusha and Kilimanjaro Regions) showing the administrative boundaries of wards within these regions were downloaded from the website of the Tanzanian National Bureau of Statistics. The data used were as published for the 2012 National Census. The ward level shape files were imported into QGIS, and merged with accompanying data on the classifications of each ward into one of three study settings. These setting classifications were developed for the Bacterial Zoonoses project and wards were classified into three settings: pastoral, agro-pastoral and peri-urban (see section 2.2.2 for more detail). ELISA result data for both the *Leptospira* serovar Hardjo and *Coxiella burnetii* were also manipulated and plotted using QGIS (see Chapter Three). For each study ward, the total number of livestock tested was represented by size of the pie chart and the proportion of animals that were positive and negative expressed by colour separation of the pie chart. Separate maps were developed for each livestock species and for each test. For example, there is one map for *Leptospira* serovar Hardjo showing the data for cattle and three maps for the data *Coxiella burnetii* showing the results for cattle, sheep and goats separately (see Chapter Three).

2.2.7 Statistical analysis

The ELISA data set was checked for errors that may arise due to missing values, and inconsistency. All statistics was performed using the R programming language (R Core Team, 2014). The outcome or dependent variable in all analyses was the serostatus of each individual animal, which was a binary outcome (ELISA positive or negative). Generalized linear mixed models (GLMMs), using the ('lme4' package) in R, were used to examine associations between this dependent variable and several host and environmental variables considered as independent variables (fixed effects). The fitted Binomial family models with a logit link function included a random effect term to account for household level variation. P values < 0.05 were considered statistically significant.

For the cattle sampled in the cross-sectional survey, the random effect variable was household, while the variables (fixed effects) screened during univariable analysis were:

- (a) Animal husbandry system/setting; a 3 level factor: Pastoral, Agropastoral or Peri-Urban.
- (b) Age of animal; a 5 level factor: 1_Temp; 2_2T, 3_4T, 4_6T, 5_Full, 6_Fullworn.
- (c) Breed; a 3 level factor: Indigenous, Cross-breed, Exotic.
- (d) Sex; a 2 level factor: Male and Female.

For cattle sampled at abattoirs, analyses were performed on sex and animal region of origin only using generalized linear models (GLM) since no random effect variable was available. All animals were adults and of indigenous breed. Model building for the cattle sampled at households and at abattoirs was performed separately.

For each cattle population, univariable analysis was performed, followed by variable selection and then multivariable analysis. For the univariable analyses, likelihood ratio tests (LRT) were used to compare each univariable model with the null model that is nested. Variables with a p-value < 0.2 in the LRT were selected for inclusion in the final multivariable model. The multivariable model was reduced by sequentially removing variables that had the highest (non-significant) p-value in LRTs comparing the current model to the set of models with one additional variable removed. This process was repeated until all variables remaining in the model had a significant p-value (<0.05) in the LRT (e.g. when the inclusion of all remaining variables significantly improved model fit). The coefficient values in the final multivariable models and the relevant univariable models were compared to

identify any problems of collinearity. Two-way interactions between main effects were also examined and significance was declared at $p < 0.05$.

2.3 RESULTS

2.3.1 Summary statistics and variable summaries for cross-sectional data

ELISA results were generated for a total of 429 cattle, sampled in the cross sectional study. Summary information for the sampled animals and the full details of the classification and number of animals sampled are shown in Table 4, while detail of the study sites where livestock were sampled are indicated in Figure 3. In total 50 of the 429 (11.7%) samples tested from this population was ELISA positive.

Figure 3: Map of study sites for the Bacterial Zoonoses project

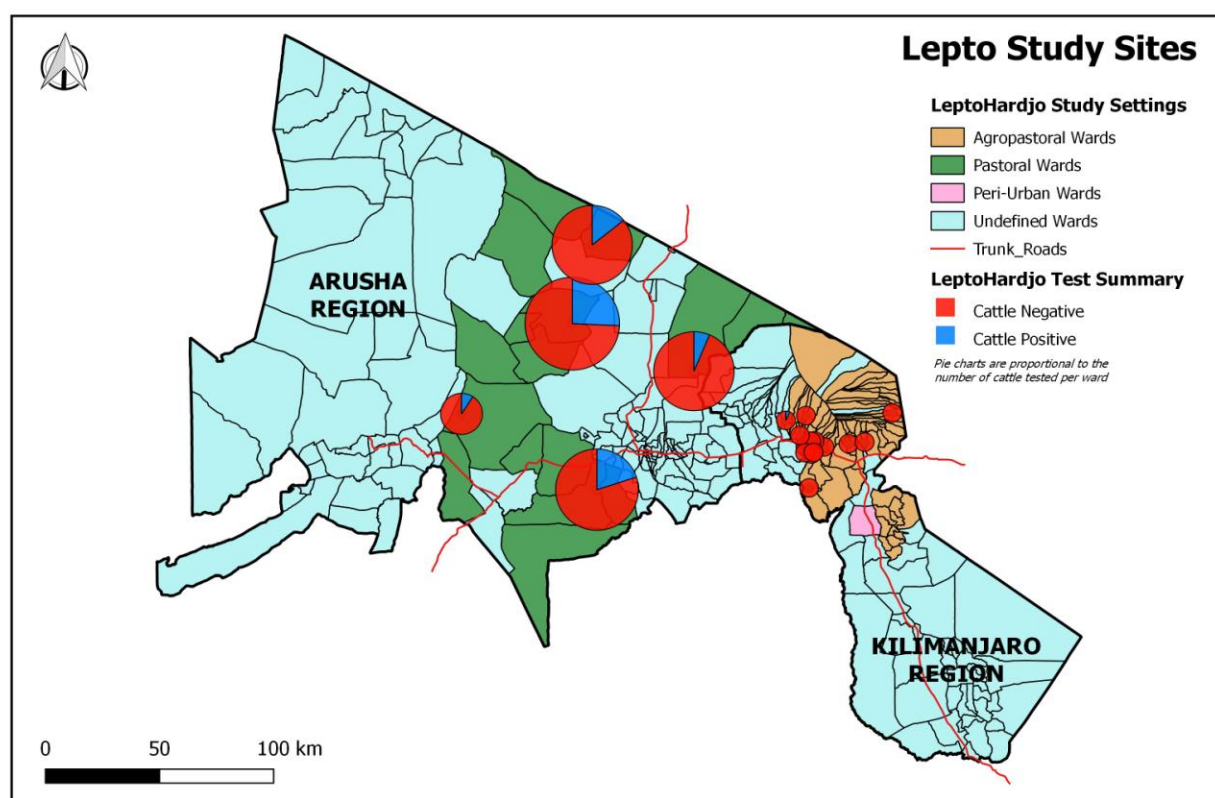


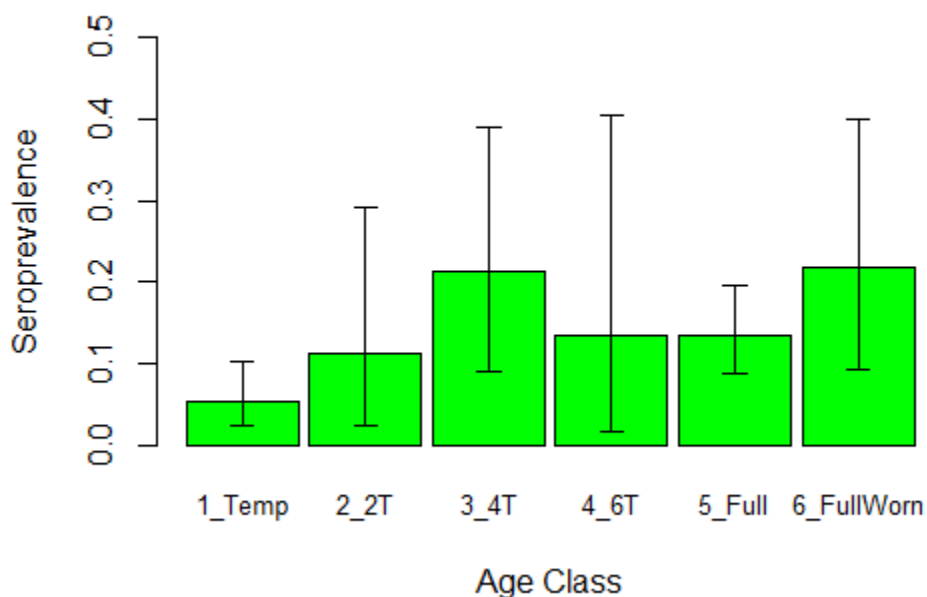
Table 4: Description of animals for which samples were ELISA tested from the cross-sectional study

Variable	Category (months)	Number Sampled	% sampled
Age	<12	152	35.4
	12-24	27	6.3
	24-36	33	7.7
	36-48	15	3.5
	48-60	170	39.6
	>60	32	7.5
Setting	Agro-pastoral	70	16.3
	Pastoral	302	70.2
	Peri-urban	58	13.5
Breed	Cross-breed	131	30.6
	Indigenous	281	65.7
	Exotic	16	3.7
Sex	Male	114	26.5
	Female	316	73.5

2.3.2 Seroprevalence of bovine *Leptospira* serovar Hardjo in the cross-sectional study

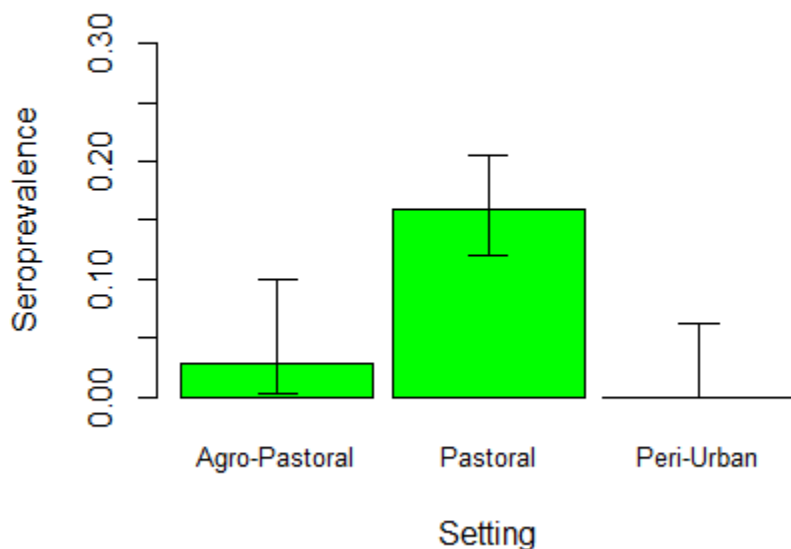
Age: The raw proportion of animals ELISA positive varied with age (Figure 4), ranging from 5.3% (8/152) in the youngest age group (with temporary incisors estimated to be less than one year of age), and increasing to 21.9% (7/32) in the oldest age class (animals with a worn set of molars estimated to be > 5 years of age).

Figure 4: Figure showing seroprevalence by age class as determined by dentition, vertical bars represent 95% confidence interval



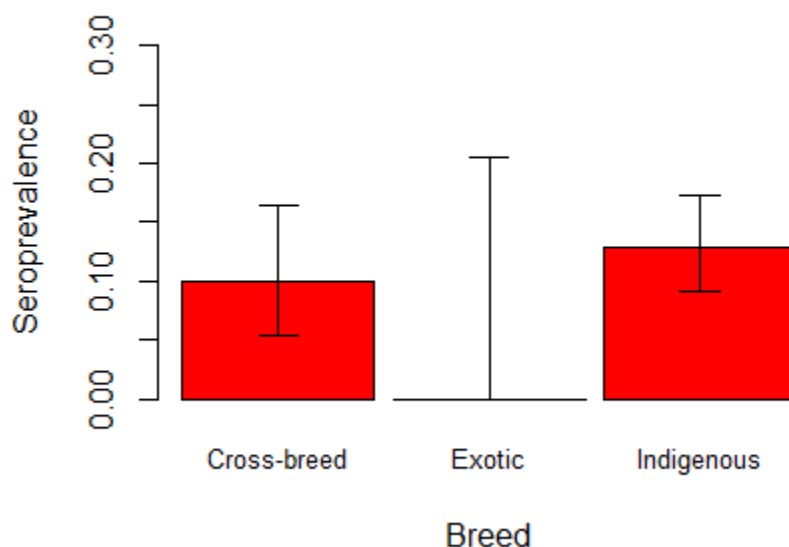
Setting: The seropositive rate observed was also different across agro-ecological settings (Figure 5), ranging from 0% (0/58) in peri-urban settings, to 15.9% (48/302) in pastoral systems.

Figure 5: Figure showing seroprevalence by setting, vertical bars represent 95% confidence interval



Breed: Seroprevalence was higher for indigenous breeds (12.8%, 36/281) (Figure 6) than for cross-breeds (9%, 13/131). There were no positive animals among the exotic breed (0/16). One of the two cattle that had missing data on their breed classification tested positive.

Figure 6: Figure seroprevalence by breed, vertical bars represent 95% confidence interval



Sex: There were more seropositive female cattle 13.3 % (42/316) than male 7.0% (16/114)

2.3.3 Univariable GLMM analyses and LRT for cross-sectional survey

The results of the likelihood ratio tests of the univariable models indicated significant associations between cattle serostatus and age (LRT: $p=0.04$) and between cattle serostatus and setting (LRT: $p=0.000$). There was no evidence from the univariable models of a significant effect of breed (LRT: $p=0.223$) or sex (LRT: $p=0.05$) on cattle serostatus.

A large standard error was observed for the coefficient estimate for the setting variable subcategory peri-urban. This was likely because there were too few observations in this category to allow robust estimation of the coefficient or to test for any statistical significance as no animal had tested positive in the region. To enable inclusion of this variable in the multivariable modelling the variable categories peri-urban and agropastoral were combined to

form a new single factor level of non-pastoral. Thus, the setting variable that was used in the model had two categories: pastoral and non-pastoral. The same process was followed for breed because none of the exotic breed tested positive and a large standard error was also observed in the model for this factor level coefficient. The categories cross-breed and exotic breed in the breed variable were combined to form a new category- non-indigenous breed. Therefore, the breed variable used in the model has two categories: indigenous and non-indigenous. The univariable analysis and LRT testing was repeated for these reformatted variables. The association between cattle serostatus and the simplified setting variable remained significant (LRT: $p=0.000$). The association between cattle serostatus and breed remained not significant (LRT: $p = 0.214$).

2.3.4 Multivariable analysis for cross-sectional survey

The variables included in the multivariable modelling were Setting, Age, and Sex (Table 4). After adding all these variables to the model and the rigorous stepwise model selection process completed, only Setting, and Age were significant and remained in the final model (Table 5). The variable Sex was not significant, and when dropped, the overall model parameters estimates became improved (Table 5). Likelihood ratio test indicated no significant interaction of Age and Sex in the final model (LRT: $p=0.291$). In addition, a LRT that further compared the models with Sex and without Sex, indicated that the simpler model without sex, is a better fit (LRT: $p=0.625$). No evidence of collinearity was observed in the final model.

The final model shows that the probability of cattle testing positive for antibodies against *Leptospira* serovar Hardjo varies across age groups. Animals of 24-36 months old were significantly ($p=0.010$), OR=4.6, 95% CI (1.4-14.4) more likely to test positive when compared with the reference group (<12 months). An evidence of association was also observed between cattle of 48-60 month old ($p=0.020$), OR=2.8, 95% CI (1.2-6.7), and the oldest animals of more than 60 months old ($p=0.010$), OR=4.7, 95% CI (1.5-14.9), as they were significantly more likely to be ELISA positive than animals in the youngest age group. Cattle of 12-24 months old have 2.6 (95% CI 0.6-11.7), times the odds of *Leptospira* serovar Hardjo seropositivity compared to animals in the youngest age group, but the observed increase was not significant ($p=0.198$). Similarly, animals in the age group 36-48 months have

4.5 (95% CI 0.7-27.0) times the odds of testing positive compared to the reference group with youngest animals, but observed difference was also not significant ($p=0.100$). Cattle in the pastoralist system of livestock production were significantly ($p=0.001$), OR=12.2 (10.3 – 13.2) more likely to test positive against *Leptospira* Hardjo, when compared to cattle from the non-pastoralist setting.

Table 5: Summary of the final model

Variable	Category(Months)	Estimate	SE	z-value	p-value*	OR (95%CI)
Age						
	<12	-	-	-	-	-
	12-24	0.9868	0.7523	1.312	0.190	2.6(0.6-11.7)
	24-36	1.5237	0.5930	2.570	0.010	4.6(1.4-14.4)
	36-48	1.5044	0.9136	1.647	0.100	4.5(0.7-27.0)
	48-60	1.0321	0.4443	2.323	0.020	2.8(1.2-6.7)
	>60	1.5456	0.5893	2.623	0.009	4.7(1.5-14.9)
Setting						
	Non-pastoral	-	-	-	-	-
	Pastoral	2.5000	0.7505	3.331	0.001	12.2(2.8-53.2)

**P-value indicates the level of significant difference for each category when compared to the reference group for the variable; SE-standard error; OR-odd ratio; CI-confidence interval.*

2.3.5 Summary statistics and variables summaries for abattoir data

A total of 93 ELISA results were generated from cattle sampled in the abattoir survey. Detail for the cattle sampled and classification of the number of animals sampled are shown in Table 6. Overall, 24.7% (23/93) of the serum samples screened were ELISA positive. All the abattoir animals sampled were adults and indigenous breeds. More male than female cattle were sampled and the majority of animal included in the survey were from Manyara region (Table 6). There was essentially no difference in the proportion of male and female animals that were seropositive [male=23.5 % (17/72), female=23.8 % (5/21)]. The seropositivity status of slaughtered cattle differs across regions of primary origin. Seropositive cases were seen most in cattle that were reported to have come from the Singida region 31 % (5/17), followed by Manyara 27.2 % (15/55) and Arusha 22.2 % (2/9). There were no positive animals from the Dodoma region (Table 6).

Table 6: Description of animals sampled in the abattoir survey for which samples were screened for *Leptospira* serovar Hardjo

Variable	Category	Number sampled (%)	Number positive (%)
Sex	Male	72 (70.0)	17 (23.5)
	Female	21 (22.5)	5 (23.8)
	Missing data	7 (7.5)	
Region	Manyara	55 (59.1)	15 (27.2)
	Singida	16 (17.2)	5 (31.3)
	Arusha	9 (9.7)	2 (22.2)
	Dodoma	6 (6.5)	0 (0.0)

2.3.6 Univariable GLM analyses and likelihood tests for abattoir data

The likelihood ratio tests of the univariable models showed no significant association between the sex and the serostatus of slaughtered cattle (LRT $p=0.671$) (Table 7). There was also no significant effect of region of origin on the serostatus of cattle sampled in the abattoir (LRT $p=0.861$). There were no positive cattle from Dodoma region (Table 6), which cause an inflated standard error in the univariable model for region of cattle origin. Cattle from Dodoma were excluded from the univariable model for region (Table 7).

Table 7: Summary of two separate univariable analyses for Region and Sex variables of the abattoir data

Variable	Category	Estimate	SE	z-value	p-value*	OR (95%CI)
Region of origin	Arusha	-	-	-	-	-
	Manyara	0.175	0.853	0.206	0.837	1.19 (-0.48-2.86)
	Singida	0.464	0.966	0.480	0.631	1.59 (-0.30-3.48)
Sex	Female	-	-	-	-	-
	Male	0.579	0.579	0.420	0.675	1.78 (0.64-2.92)

*P-value indicates the level of significant difference for each category when compared to the reference group for the variable; SE-standard error; OR-odd ratio; CI-confidence interval.

2.3.7 Multivariable analysis for abattoir survey

The variables considered in the model for abattoir survey had a $p>0.02$ in the univariable LRTs. Multivariable analysis was not performed for cattle sampled in the abattoir survey.

None of the variables considered in the study were significantly associated with cattle *Leptospira* serovar Hardjo ELISA status.

2.4 DISCUSSION

This study provided evidence of widespread infection of cattle with *Leptospira* serovar Hardjo in northern Tanzania. The ELISA data from the cross-sectional study population demonstrate statistically significant variation in infection prevalence in different livestock production systems and in different age classes. The highest seroprevalence (16%) was observed in cattle from pastoral farming systems, while no evidence of infection was found in cattle kept in peri-urban systems. The age of cattle increases the chances of testing positive for leptospirosis. However, the variation in prevalence across age groups was not consistent or linear. While this may suggest differences in the level of exposure for each age group of animal in the livestock population, it is also possible that the relatively small number of animals in some age groups could have affected the observations. The overall prevalence of *Leptospira* serovar Hardjo infection in this study (12%) was broadly consistent with data from elsewhere in sub-Saharan Africa. Previous studies that had used similar laboratory diagnostic methods (e.g. ELISA for serovar Hardjo), reported infection prevalences varying between 10% and 35% for *Leptospira* serovar Hardjo in different regions of sub-Saharan Africa (Ngbede et al., 2012c; Ngbede et al., 2013; Scolamacchia et al., 2010). In addition, two recent studies that were conducted in Tanzania, but had used the MAT as the laboratory diagnostic methods, reported similar prevalence values of 15% (98/655) for *Leptospira* serovar Hardjo in cattle from small farm holders that are extensively managed in the Tanga region (Schoonman and Swai, 2010), and 17.6% (194/1104) in cattle from Katavi region (Assenga et al., 2015). The serological studies that used MAT may not be directly comparable with the results of this study. However, these data still indicate a similar finding on the seroprevalence of leptospirosis in cattle in Tanzania. The laboratory diagnostic methods used as well as many features of the tested populations (e.g. animal husbandry systems, seasonality and environmental factors such as climate change), may account for the wide variation in seroprevalence of bovine leptospirosis reported from these different study populations. Animals from the smallholder (agropastoral) and peri-urban systems showed lower infection levels (3.0%), and these findings are more consistent with data from Asia where prevalences of 3% - 6% have been reported (Aisser et al., 2013; Kocabiyik and Cetin, 2004).

Given the complexity of leptospirosis epidemiology, several factors have the potential to affect infection risk and to contribute to the observed variability in infection prevalence

across regions. In recent years, very few cross-sectional studies have been carried out to identify and evaluate factors that are associated with the seroprevalence of bovine leptospirosis in northern Tanzania. In this study, cattle in the pastoral systems of livestock farming were significantly more likely to test positive to serovar Hardjo, when compared to non-pastoral systems. The finding of higher seropositivity of *Leptospira* infection in pastoral systems is consistent with findings from other studies in this region, where pastoral systems were also a significant risk factor for higher levels of infection with *Mycobacterium bovis* (Cleaveland et al., 2005), and foot and mouth disease virus (Lembo et al., 2012). There are several features of pastoral systems that may pre-dispose to high levels of infection. These systems are typified by large herd sizes, wide-ranging grazing systems, high levels of mixing with cattle from other herds, as well as contact with small ruminants, potential exposure to wildlife populations, and generally low levels of bio-security. In addition to these characteristics, the pastoral system also has a high rate of acquisition of new animals that would increase the chances of introducing new infections as have been demonstrated in foot-and-mouth disease transmission dynamics (Casey-Bryars, 2016).

Large herd size has specifically been identified as a key risk factor for other infectious diseases of livestock in pastoral systems (Casey-Bryars, 2016), and may also be an important determinant of *Leptospira* infection. This might be expected as there is a greater chance of an infected cattle being present in a large herd than in a small herd, and therefore a greater potential for transmission to other individuals through environmental contamination and/or direct contact. Also, large herd size may be associated with extensive animals farming systems without any control on animal breeding, thereby increasing other transmission pattern of leptospirosis such as sexual transmission and maternal or vertical transmission. An alternative explanation relates to extinction rates; in large herds the probability of infection becoming extinct as a result of stochastic factors is likely to be lower than in small herds (Cleaveland et al., 2005). Whatever the underlying mechanism, the finding of an increased prevalence in large herds suggests that within-herd transmission routes are likely to be important factor in the prevalence of leptospirosis in pastoral systems of cattle farming.

Leptospira serovar Hardjo has also been identified among the predominant circulating serovars in buffaloes (Assenga et al., 2015; Hajikolaei et al., 2006; Kenar and Ozdemir, 2013). The potential exists for contact between buffaloes and cattle in Monduli District, where pastoral herds were sampled in this study, which is possible through sharing of

drinking water sources. However, it is unclear the degree to which this may be occurring and the significance of wildlife-livestock transmission in the epidemiology of the disease. For other diseases, it has often been assumed that wildlife-livestock transmission is important, but epidemiological data have not supported this assertion. For example, cattle management factors have been shown to be much more important risk factors for FMD (Casey-Bryars, 2016; Di Nardo et al., 2011; Vosloo et al., 2004) and bovine TB infection (Munyeme et al., 2008) than wildlife factors, such as the degree of contact with wildlife or proximity to wildlife-protected areas.

It was also observed that seropositivity levels were highest in areas of lowest rainfall i.e. the semi-arid rangelands of the pastoral systems. Flooding has often been identified as a risk factor for leptospirosis in humans (Bhardwaj et al., 2008), and possibly increase transmission risk to animals. This suggests that the nature of rainfall in predominantly dry systems (which is short lived but intense) does lead to flooding at times, and transmission opportunities may increase when animals aggregate at these pools (watering points), which are also likely to be contaminated with leptospires.

The observed significant difference in seropositivity for *Leptospira* Hardjo between age groups of cattle in both the univariable and multivariable models was consistent with other similar studies that have reported an increase in exposure levels with age (Aisser et al., 2013; Kocabiyik and Cetin, 2004; Ngbede et al., 2012c). This is also consistent with an interpretation of endemic infection where the probability of becoming exposed will increase with age. Therefore, for pathogens where antibody titres are long-lived, the older an animal gets the more chances of testing positive. Even where antibody titres may wane, seropositivity in older animals is likely to be detected in an endemic setting, as the chances of repeated exposure and boosted immune response are also more likely to be seen in older animals. The higher likelihood of repeat exposure and boosted immune responses in older animals may also be an explanation for clinical signs being less severe in older animal than in younger and naïve animals where the disease can be fatal.

While there was a strong effect of age, there was no consistent trend of increasing positivity with age, with particularly high levels of infection detected in the 3-4 year age class. It is therefore possible that there may also be differences in exposure with age and/or differences in the rate of decline of antibodies in different age classes. One possible explanation relates to

exposure through sexual transmission, where exposure levels increases with animals reaching sexual maturity (Picardeau, 2013).

The finding of zero seroprevalence in exotic breeds may not necessarily reflect innate breed differences, and is likely to have been confounded by farming system, with the exotic breed individuals in this study all coming from small herds in peri-urban or smallholder agro-pastoral herds. Furthermore, breed and sex were not significant in our univariable analysis even after the breed variable was transformed. This is consistent with studies reported elsewhere in West Africa (Ngbede et al., 2012a), which observed a significant difference with age of animals but not in breed and sex. A study on the prevalence of leptospirosis in buffaloes also found no difference in the sex of the animals (Kenar and Ozdemir, 2013).

This study investigated the seroprevalence of *Leptospira* serovar Hardjo in cattle populations in two different systems: a cross-sectional study and an abattoir survey. The prevalence of exposure in cattle sampled at abattoirs (25%) was higher than those sampled in the cross-sectional study (12%), and higher even than cattle sampled in pastoral systems (16%). This may reflect differences in infection levels at different sources of cattle origin since animals are brought to these abattoirs from different regions. In the abattoir survey, cattle presented for slaughter originated from two main regions: Singida region (17.2% animals) and Manyara region (59.1% animals), and the prevalence of exposure in these regions, though not significant, were also highest for cattle from these two regions. The seroprevalence observed in cattle originated from the Singida region (predominantly pastoral) was 31.3%, and 27.2% seroprevalence from the Manyara region (mostly agro-pastoral). Since antibodies are likely to be detected from 10-14 days post exposure (Musso and La Scola, 2013), it is likely that the seroprevalence in these cattle reflects higher levels of infection acquired either at their household of origin or during transit to the abattoir, rather than at the abattoir, as has been previously suggested as explanation for high infection prevalence in abattoir cattle in Texas (Talpada et al., 2003). This is because in typical sub-Saharan market systems, slaughtered cattle are unlikely to stay at the abattoir for as long as 10-14 days, which is the time required from exposure to presence of antibodies in the blood (Musso and La Scola, 2013). An informal interview with market officials conducted during a personal visit to the Weru Weru cattle market (a secondary market near Moshi where the slaughtered cattle were sampled) in January, 2016, confirmed that Manyara and Singida are the two major sources of cattle supply to the market. Information gathered in the visit suggests that cattle can mix for more

than a week in the market channels before being slaughtered at the abattoir. This is particularly likely when a potential buyer is not found on a market day, and the cattle are returned several times to the market or are kept in mixed grazing fields several days to weeks before being sold or resold.

Another possible explanation for the higher seroprevalence seen in abattoir cattle as compared to the cross-sectional populations is that owners are preferentially selecting infected animals for slaughter. This may be done on the basis of poor productivity such as infertility, abortion and/or reduced milk production, which are major signs of bovine leptospirosis. A similar process has previously been proposed as an explanation for the finding of a disproportionately high prevalence of *Mycobacterium bovis* infection in slaughtered cattle in the same regions of Tanzania when compared to infection levels in cattle sampled through cross-sectional surveys (Cleveland et al., 2005).

The differences in seropositivity in male (7%) and female (13%) cattle in the cross-sectional study as compared to the abattoir survey (23% for both sexes), further supports the suggestions that market networks may be a major source of exposure of cattle to leptospirosis especially in endemic settings where the disease is prevalent, vaccination not practiced and market dynamics such as the Weru Weru markets encourages potential mix of male and female cattle over several days or weeks. It is also possible that cows (with probably higher seroprevalence levels), may have been selectively sent to slaughter on the basis of poor productivity and illness, but had then mixed with male cattle in the market channels leading to similarity in seroprevalence at the abattoir.

A prevalence of 25% in the abattoir survey is quite high considering it is for *Leptospira* serovar Hardjo only. The public health significance of this is huge. The abattoir pose a major risk for human transmission because the urine contamination during slaughter is very common and circulating serovars in cattle are also among those that affect humans (Assenga et al., 2015). There are also no safety measures in the abattoir in most developing countries to help prevent direct contact with animals' products at slaughter, which indicates that abattoir workers, butchers, and animal handlers are at high risk of infection. It is common to see butchers using bare hands and walking with bare feet at the abattoir. Introducing sensitization programmes to encourage the use of protective clothing materials in handling animal products during slaughter among abattoir workers will help to reduce the risk of exposure to the pathogen at this high-risk groups.

Reducing the burden of bovine leptospirosis at the farm levels is critical to reduce the burden of the disease in the high-risk human populations such as farms workers and would also reduce the number of exposed or sick cattle that end up in the abattoir. Bio-safety measures that can be targeted at farm levels such as vaccination, improved diagnostic techniques, quarantine of sick animals, restriction of animal movements, treatment and general farm hygiene have been effectively used to control leptospirosis in most developed countries (Menges, 1959; Ryan et al., 2012). This has not been the case in most low resource countries such as Tanzania (Assenga et al., 2015; Schoonman and Swai, 2010) because the cost of vaccination and treatment seems to be a huge burden to farmers without government subsidies. The prevalence of the disease remains high as observed in this study. Control measures such as vaccination, diagnosis and treatment, and farm hygiene can be effectively used to control leptospirosis particularly in the peri-urban and smallholder agro-pastoral with intensive and semi intensive systems of husbandry.

Factors such as socio-economic status and cultural practices as regards to livestock keeping, which affect relevant control measures, are different in the peri-urban, smallholder agro-pastoral and pastoral settings. The differences in terms of awareness of the disease and available prevention and control options for each production systems may also affect control strategies. The Maasai pastoralists' communities in East Africa for instance have unique social and cultural values by which they associate with their animals, and may be willing to uptake vaccination and treatment for their livestock if they aware about the benefits, and if it is accessible and affordable. However, other biosafety measures such as quarantine, selective breeding techniques and farm hygiene may not be practicable in the pastoral systems because of migratory herds.

Livestock production and performance are the major problems that have been associated with bovine leptospirosis. It causes infertility and reduced milk yield, which are very critical to farmers due to production losses. There are currently no available data on the true impact of these losses to farmers and the implications cannot be determined. This makes it very difficult to instigate government to introduce or initiate policies that can help to reduce the burden of the disease in animals and humans in Tanzania and other sub-Saharan countries. In addition, limited resources in endemic countries restrict most government to undertake a comprehensive disease control programme. The decision makers in these countries would require a trade-off between cost and benefits, especially when so many diseases are endemic,

before engaging in any disease control. Policy makers often do not consider leptospirosis a disease of high impact in livestock production when compared to FMD or highly pathogenic Avian Influenza (Knight-Jones and Rushton, 2013) and the disease may likely remain neglected in poor countries in the near future until the true impact is fully appreciated. Infected cattle that become carriers can continue shedding the pathogen to the environment and infect other cattle (Pedersen et al., 2015). This becomes more critical for farmers especially at herd levels because the disease is maintained in the farms with more consequences.

The findings of the highest seropositivity of leptospirosis in pastoralist settings present a further complication to the livestock industry in Tanzania because pastoralists usually take their cattle to regional cattle markets for sale. Since there is no adequate evaluation of cattle before purchase, unsuspecting buyers can easily buy apparent healthy animals that have been exposed and reintroduce them into their own herd for breeding. This is a very common practice because the cattle markets are the major source of supply to both butchers and other small farmers. Thus, the spread of the disease is increased and maintained in the region. Animals that may not have been exposed, can potentially become infected when mixing with sick animals in the market channels, and subsequently present a risk to humans at slaughter or to other farms when sold. Age of cattle as a significant factor we observed in this study is also of economic relevance to the farmers. Leptospirosis can be transmitted vertically from parent to offspring (Levett, 2001). This means older animals will not only shed the pathogens in the environment, but can also transmit to newborn.

Although cattle are susceptible to several serovars of *Leptospira*, seroprevalence studies have shown that Hardjo is predominant among the major circulating types in Tanzanian cattle (Assenga et al., 2015; Schoonman and Swai, 2010). In this study, the serological test used was specific to *Leptospira* serovar Hardjo, and the results support the view that serovar Hardjo is a prevalent circulating serovar in Tanzanian cattle. *Leptospira* serovar Hardjo could pose a potential risk for human transmission especially those in contact with livestock.

In conclusion, *Leptospira* serovar Hardjo is endemic in cattle with high seroprevalence in northern Tanzania. The pastoralist system of animal husbandry is a major driver of the seroprevalence of the disease and older animals are more likely to be seropositive. The high prevalence has the potential for major impacts on productivity and performance of cattle,

and poses a threat to public health due to human transmission from cattle. Improving the general awareness of the diseases in high-risk populations will be crucial for effective control and prevention in humans and their animals.

CHAPTER THREE

Exposure patterns of Q fever within livestock populations in Northern Tanzania

3.1 INTRODUCTION

Q fever is an important zoonotic disease of global public health significance. It affects man, domestic and wild animals worldwide (Maurin and Raoult, 2010), and is prevalent in both developed and developing countries with the exception of New Zealand (Hilbink et al., 1993; Huebner and Bell, 1951). The disease is characterised by both acute and chronic infections in livestock causing huge production and performance losses due to large scale abortion, and contributing to infertility problems in herds (Angelakis and Raoult, 2010; Tissot-Dupont and Raoult, 2008). The derived name ‘Q’ fever, originated from ‘Query’ fever named after an outbreak of unidentified febrile illness among abattoir workers in Brisbane, Queensland Australia, in 1935 (Derrick, 1937). The disease was called Query fever apparently, due to the difficulty in identifying the causative agent. It was much later that some of the workers suggested that in the adopted name ‘Q fever’, Q stood for Queensland, the state where the disease was first reported (McDade, 1990).

Coxiellosis in animals and Q fever in humans is caused by a bacterium, *Coxiella burnetii*. The genus *Coxiella* has only one species: *burnetii*. *C. burnetii* is a highly infectious agent that has been identified as a potential candidate for bioterrorism, prompting its classification as a group B pathogen by the Centre for Disease Control and Prevention (CDC), in the United States (Tissot-Dupont and Raoult, 2008). It was first isolated in 1937 from experimental guinea pigs that have been inoculated with urine from infected human patients who had contracted the infection in an abattoir (Derrick, 1937). It was originally classified as a rickettsial pathogen called *Rickettsia burnetii* (Burnet and Freeman, 1937). However, in 1948 Philips described the pathogen to be similar in morphological and biochemical characteristics to other gram-negative bacteria (Philip, 1948). He subsequently classified the pathogen to a new genus, *Coxiella*, after Herald R. Cox, who had first isolated the bacteria in the USA (Philip, 1948). These findings, and the new classification have subsequently been substantiated by phylogenetic analysis based on 16S rRNA sequence data that confirms that the genus *Coxiella* is distinct from the genus *Rickettsia* (Drancourt and Raoult, 2005).

Until recently, Q fever has been relatively unknown and underappreciated as a cause of human and animal disease, notwithstanding its OIE notifiable disease status. Renewed interest and awareness of the disease in developed countries has been provoked by the largest recorded outbreak of the disease in history. This outbreak occurred in the Netherlands during 2007-2010; 2357 human cases were reported in 2009 (Hoek et al., 2010), and by 2010 over 4000 cases have been reported (Delsing et al., 2010). The factors leading to this outbreak were not fully understood initially. However, repeated waves of abortions amongst farmed dairy goats were identified as the primary source of human exposure, and living within a 5km radius of an affected farm was among the major risk factors identified for human infection (Roest et al., 2011). This confirms suggestions that inhalation of aerosolised bacteria particles is among the main route of humans exposed to the pathogen (Angelakis and Raoult, 2010; Raoult, 1996). In addition, ingestion of contaminated milk, direct contact with infective faeces, urine and birth tissues, are also potential routes of infection (Raoult, 1996).

Information on the current situation of Q fever in Africa is very scarce. Some of the few outbreaks that have been reported were in East Africa, in 2000 (Potasman et al., 2000) and 2014 (Kenya Zoonosis Diseases Unit, 2014), among safari travellers and rural village dwellers respectively. Notwithstanding Q fever was first reported in Africa in 1947 (Blanc and Maurice, 1947), the epidemiology remains very poorly understood. This may be partly attributed to the poor recognition and reporting of the disease, similar to most other endemic zoonotic diseases such as leptospirosis and brucellosis (Halliday et al., 2015). Early studies conducted in Kenya, suggest that Q fever has been widely misdiagnosed as malaria and viral pneumonia (Brotherston and Cooke, 1956; Craddock and Gear, 1955), and more recently in Tanzania, it was also reported that Q fever among other zoonotic pathogens was misdiagnosed as malaria (Crump et al., 2013). These cycles of misdiagnoses may have contributed to poor awareness, reporting, and under estimation of the disease burden in Africa (Vanderburg et al., 2014).

Coxiellosis in livestock, although highly prevalent in sub-Saharan Africa (Knobel et al., 2013; Scolamacchia et al., 2010), is poorly recognised and under reported in animal populations (Njeru et al., 2016). The epidemiology of livestock coxiellosis is still relatively unknown. It has become a neglected disease in low income countries because, although it is highly prevalent (Knobel et al., 2013), there is a lack of information about the impact of coxiellosis on livestock production, its zoonotic potential and policies for the surveillance, prevention and control (Njeru et al., 2016). Because of this historical neglect, the disease remains highly

endemic and prevalent in affected communities in sub-Saharan Africa (Knobel et al., 2013; Scolamacchia et al., 2010). Control measures such as good farm hygiene, vaccination and treatment using antibiotics are available and have been used to prevent the disease in developed countries. However, this is not the case in sub-Saharan Africa, where there are no control measures being practiced and the disease remains endemic.

Livestock coxiellosis has never really been studied in Tanzania, which appears to be the case for most other sub-Saharan African nations (Njeru et al., 2016; Vanderburg et al., 2014). The absence of reliable data on the prevalence of coxiellosis across livestock species severely hinders the understanding of the spread of infection and disease burden. In addition, the knowledge of the exposure patterns in livestock may be important in assessing likely sources for human infection especially in closely epidemiologically linked human and livestock populations that are commonly seen in developing countries.

3.1.1 Host range, reservoirs and prevalence

Coxiella burnetii has been isolated from arthropods and birds, but the livestock species: cattle, sheep and goats, are considered the main reservoirs of infection in both animals and humans (Babudieri, 1959; Maurin and Raoult, 2010). The reservoir status of different host species for *C. burnetii* may vary across geographical regions. This is because, while livestock are considered the main reservoirs of infection in Africa and other developing countries, cats were identified as the major reservoirs of infection during a Q fever outbreak in Nova Scotia, Canada (Marrie et al., 1988).

A review of coxiellosis in Africa indicated that acute and overall seroprevalence of infection tends to be higher in small ruminants as compared to cattle in the northern (Egypt) and southern (South Africa) countries, but this pattern was not consistent across Africa (Vanderburg et al., 2014). A more recent review in Kenya also identified very few studies with appropriate designs that had compared species prevalence in linked livestock populations (Njeru et al., 2016), and the current prevalence in livestock populations in sub-Saharan Africa remains largely unknown. In humans however, the overall prevalence in Africa varies, ranging from 1% in Chad to 16% in Egypt, among humans that were considered to be in close contacts with animals such animal handlers and abattoirs workers (Vanderburg et al., 2014).

3.1.2 Transmission of *C. burnetii* in livestock populations in endemic settings

Coxiella burnetii is well adapted for survival outside animal hosts. When free living in the environment, it develops an infectious spore-like form that can survive for several months (Drew, 2004; McCaul, 1991). This allows the spread and persistence of the pathogen within livestock populations even after long periods of presumed absence. It is also resistant to harsh weather conditions such as extreme dryness, cold, heat and disinfectants (McCaul, 1991). These properties of the pathogen are likely to be the reason for its relative abundance in both tropical and temperate conditions.

A review of epidemiological and experimental evidence suggests that transmission in livestock populations can occur via several routes, including through tick bites (Stoker and Marmion, 1955), ingestion, and by direct contact with infective materials such as birthing tissues and fluids (Sanford et al., 1994), sexual route, and inhalation of aerosolised *C. burnetii* particles (Angelakis and Raoult, 2010). *C. burnetii* has been isolated in ticks found on livestock hosts, and had been assumed to play an important role in the transmission of the bacteria among livestock species especially when favourable ecological and agricultural factors that encourage contacts between ticks and livestock are present (Stoker and Marmion, 1955). Even though this transmission route is possible, it is not considered essential in the natural infection cycle in livestock (Babudieri, 1959). Oral transmission, by ingestion of infected water and feed materials is possible especially when contaminated with infective milk and birth tissues (Sanford et al., 1994). The isolation of an active form of the pathogen from bull semen suggests that sexual transmission, which has been demonstrated experimentally in mice (Kruszewska and Tylewska-Wierzbanska, 1997, 1993), is also possible. However, oral, tick bites, and sexual transmission modes appear to have been less explored.

Inhalation of contaminated air is considered the main mode of transmission of *C. burnetii* in livestock populations (Tissot-Dupont et al., 1999). Transmission occurs when susceptible animals inhale materials containing the bacteria as dust particles. The aerosolised bacterium can also spread by wind to nearby herds which is possible because the pathogen can survive for weeks outside animal hosts (Tissot-Dupont et al., 1999). A typical case of transmission by inhalation was in the outbreak in the Netherlands where more than 4000 human cases were reported (Delsing et al., 2010). The persistence of the *C. burnetii* in the environment, and

potential transmission through inhalation could frustrate efforts in identifying sources of infection in an endemic area especially during an outbreak, which would also further complicate prevention and control options.

Direct and indirect transmission has have also been reported. An example of direct transmission was reported during an investigation into a *C. burnetii* case of abortion in a goat farm, and the primary source of the exposure was identified as a prior contact with potential infective goats from another herd that had kidded prematurely during a fair (Sanford et al., 1994). Farm contamination during parturition is also common even in areas of good farm hygiene and biosecurity Infective birth tissues are the major sources for contaminated fomites, which are vehicles for indirect transmission (Sanford et al., 1994).

In sub-Saharan Africa, where mixed livestock production systems are often practiced, it is common for farmers to keep sheep, goats and cattle all together in the same household herd or flock. In addition to mixed livestock keeping at household levels, mixing of different livestock species is also possible at communal grazing and watering points typical of agropastoral and pastoral settings in East Africa. Since *C. burnetii* infection is apparently widespread in sheep, goats and cattle (Babudieri, 1959), transmission from one livestock species to another is also very likely. The exact transmission patterns in and between livestock species have not yet been described in detail though. It is still unknown which of these livestock hosts is more responsible in maintaining the pathogen in endemic areas, and whether transmission to cattle depends on the presence of sheep and goats or vice versa. Exploring these exposure and transmission patterns will be critical in understanding maintenance and spread of the pathogen, and to enable the development of more effective control options tailored to these specific settings.

3.1.3 Clinical infection and shedding characteristics in livestock

The incubation period of *Coxiella burnetii* is highly variable in livestock, and reproductive failures may be the only indication of infection (Angelakis and Raoult, 2010). Infected animals shed *C. burnetii* in faeces, urine and milk, vaginal mucous and during parturition in birthing tissues, which mostly occurs in chronic coxiellosis (Arricau-Bouvery and Rodolakis, 2005; Dekker, 1998; Guatteo et al., 2006). While cattle and goats shed large quantities of the bacteria in milk, shedding in sheep is commonly seen in faecal and birthing materials (Table

1) (Rodolakis et al., 2007). Shedding can also occur in asymptomatic and seronegative animals (especially in cattle where it is often asymptomatic), which greatly complicates the surveillance of coxiellosis, the interpretation of serological data and the development of public health guidance. In the USA, more than 94% of bulk tank milk samples from apparent healthy cattle tested positive for *Coxiella burnetii* DNA (Kim et al., 2005). Dairy cows appear to be more infected with chronic coxiellosis than sheep and goats (Rodolakis et al., 2007). Exposed cows can continue shedding of the pathogen in milk for several months (Huebner and Bell, 1951). Epidemiological evidence suggests that exposed cows shed *C. burnetii* almost exclusively in milk (Rodolakis et al., 2007), with a very small proportion that shed the pathogen through other routes (Guatteo et al., 2006). *C. burnetii* is a highly infectious agent. One of the early studies on *C. burnetii* in the United States, in 1951, indicated that introduction of an infected cow (a cow from a herd with history of coxiellosis) into a new (previously unexposed) herd, resulted in a 40 percent of infection in the new herd within 6 months (Huebner and Bell, 1951). Infection in cattle may be asymptomatic, mild or severe. In severe cases, abortion storms, stillbirths and weak calves are often seen and are indicative of herd infection (Lang, 1990). *C. burnetii* infection in exposed pregnant cows, has been reported as cause of abortion in 11.6% (Parisi et al., 2006) to 17.2% (Clemente et al., 2009) of cattle. The infection is mostly associated with late term abortions in cattle and abortions may occur suddenly without any prior clinical indications (To et al., 1998). Metritis (in aborting cows) has also been reported to persist for several months, and is considered specific to bovine coxiellosis infections (To et al., 1998).

Table 1: Duration of shedding *Coxiella burnetii* in Livestock

Shedding route	Livestock species		
	Cattle	Sheep	Goats
Milk	13 months[a]	8 days[b]	>4 months[c]
Faeces	few days[d]	18 days [e]	>1month [f]
Vaginal mucous	rare[a]	several months[b]	several weeks[f]

a=(Biberstein et al., 1974), b=(Berri et al., 2001), c=(Berri et al., 2007), d=(Lang, 1990), e=(Marrie, 1990a)
f=(Arricau-Bouvery et al., 2003)

Goats have multiple shedding routes including milk, vaginal mucous, and birth tissues (Table 1). Shedding of *C. burnetii* in milk occurs, but most shedding is in the vaginal mucous and birth tissues during parturition (Arricau-Bouvery et al., 2003). A large quantity of the

pathogen is normally shed in goats, which can occur at subsequent kidding season (Berri et al., 2007).

Similar to cows, chronic infections lasting several months have also been reported in goats (Lang, 1990). Infected goats may show signs of depression and poor appetite 1-2 days before abortion, but reproductive failures are the obvious signs. Infertility problems, and weak kids have all been reported (Berri et al., 2007). Similar to cattle, abortion in goats is late term, and the rate is considered to be highest compared to other livestock species (Chanton-Greutmann et al., 2002; Moeller, 2001; Palmer et al., 1983). In addition to high abortion rate in goats, available epidemiological evidence suggest that abortion epidemics have been reported more in goats than other livestock species, and in overall reproductive and performance related issues are observed more in goats and sheep than cattle (Agerholm, 2013). Unlike cattle, there is no evidence to indicate metritis in goats post abortion; however, cases of endometrial inflammation have been reported after abortion, which regresses without administration of treatment (Sanchez et al., 2006).

Sheep shedding of *C. burnetii* in milk has a much shorter duration compared to goats and cattle (Table 1). Although, shedding routes (vagina mucous and birth tissues) of sheep are similar to goats, where large quantities of the bacterium can be expelled into the environment for about 4 months after parturition, and in subsequent lambing season (Berri et al., 2001; Rodolakis et al., 2007).

Experimental studies suggest that acute coxiellosis in ewes results in high fever (up to 40° C) for 2-3 days, which is associated with depression, conjunctivitis, rhinitis and interstitial pneumonia (Agerholm, 2013; Martinov et al., 1989). These signs are more pronounced with lambing and infection mostly results in weak lambs or stillbirth accompanied by inflamed placenta (Agerholm, 2013; Martinov et al., 1989). Late abortion has also been observed in ewes with an abortion rate of about 13% (Berri et al., 2005).

3.1.4 Serological response

Expression of antibody to *C. burnetii* exposure occurs in two distinct antigenic phases that are mediated by changes in the lipopolysaccharide (LPS) profile of the bacterium outer membranes (Moos and Hackstadt, 1987; Setiyono et al., 2005). Phase I antigen has a

complete LPS on the bacteria membranes and is the virulent form of the pathogen (Moos and Hackstadt, 1987). Phase II has an incomplete LPS due to loss of genetic information and is mostly avirulent (Setiyono et al., 2005). These antigenic phase variations are useful in serologically differentiating acute and chronic infections (Peacock et al., 1983). Antibody response to Phase I antigen is predominantly observed in chronic infections, while antibody response to Phase II antigen is mostly associated with acute form in humans. In animals, antibody responses to *C. burnetii* infection are mostly seen in Phase I antigen (Fournier et al., 1998). It has been demonstrated experimentally, in mice, that in acute infections diagnostic antibody titres against *C. burnetii* can be detected from day 10 post infection (Novák et al., 1992). However, data on antibody response to natural infection with *C. burnetii* in animals are very scarce. In humans, immunoglobulin M (IgM) antibodies to phase II antigens are the first to appear in early infection (from the second week) (Setiyono et al., 2005). If antibiotic drugs are not administered, the phase II antigen-specific IgM may then circulate for 7 - 15 days and peak at 4-8 weeks after the clinical signs have been observed (Peacock et al., 1983). Immunoglobulin G (IgG) antibodies appear much later and can last for several years. In chronic stages of infection diagnostic titres of phase I IgG and IgA antibodies are predominant, and is indicative of chronic infection, similar in both humans and animals (Parisi et al., 2006). The absence of a diagnostic antibody titre in both antigenic phases in livestock may not necessarily suggest absence of infection because diagnostic titres may not be visible until 10 days post infection.

3.1.5 Diagnosis of Q fever in livestock

Serology

Coxiellosis is very difficult to diagnose clinically because of its non-specific presentation in infected animals. Laboratory identification of the pathogen remains the most reliable means of detection and diagnosis. Serological methods that are available for veterinary use include complement fixation test (CFT), enzyme-linked immunosorbent assay (ELISA) and indirect immunofluorescent assay (IFA) (Porter et al., 2011). CFT had been previously used in animal science as the reference serological test, but has a major disadvantage because it utilises antibodies to phase II antigens (Krt, 2003). IFA is currently the gold standard serological test for *C. burnetii* and can detect antibodies to both Phase I and II antigens separately (Porter et

al., 2011). The IFA has been extensively used for diagnosis in humans, and can also be effectively used in veterinary medicine (Porter et al., 2011; Rousset et al., 2007). The IFA and ELISA tests have a higher sensitivity than CFT and can detect both IgG and IgM antibodies (Field et al., 2000; Fournier et al., 1998). The IFA and ELISA have also been demonstrated to have an overall good agreement when used in goat populations (Rousset et al., 2007). Positive ELISA result has been reported to have a strong correlation with abortion outbreaks in goats (Rousset et al., 2007). ELISAs are easier to perform and standardize in the laboratory and mostly available commercially (Fournier et al., 1998). Another advantage of ELISA is that it can be modified to detect antibodies to both Phase I and II antigens and automated for use in large field studies (Rousset et al., 2007).

However, these serological methods are best used to demonstrate infection or exposure at herd or flock level rather than at an individual level. Diagnostic antibody titres may not be detected by serology for the first 10 days of infection with *C. burnetii*, which suggest that serological tests such as ELISA, CFT, IFA are not likely to be effective in early infection (Kuroiwa et al., 2007). Even though seroconversion may be used to indicate recent infection, it would require a second convalescent phase serum sample to be tested at 2-3 weeks after the first sample collected in the acute phase of infection. Another limitation of using serology to diagnose coxiellosis is that seronegative animals may still be shedding the pathogen in the environment while seropositive ones are not (Berri et al., 2007, 2001). Studies that evaluated shedding characteristics of *C. burnetii*, reported that 10-20% of animals that were seronegative were shedding, which poses a serious challenge in the interpretation of serological data as seronegative may still pose a substantial risk (Berri et al., 2007, 2001; Rodolakis et al., 2007).

Antigen detection

Molecular detection of *C. burnetii* can be done using polymerase chain reaction (PCR) assays, which has shown to be very effective in early infection (Hoover et al., 1992), histology immunohistochemistry, and culture. Trans-PCR assays have been widely used in prevalence studies to detect *C. burnetii* in bulk milk tanks by targeting a transposon-like sequence found in *C. burnetii* (Kim et al., 2005; Lorenz et al., 1998). A major advantage of PCR over serology is that it detects early infection and could also detect seronegative animals that are shedding (Hoover et al., 1992); while serology requires at least 10 days post exposure and diagnostic titres may not be achieved in low shedding animals (Kuroiwa et al., 2007), which

makes the test unreliable. However, PCR is relatively expensive, requires expertise, and a negative PCR result does not necessarily rule out infection.

3.1.6 Economics of Q fever

Infection with *C. burnetii* is considered to be associated with low mortality in livestock but the morbidity can be very severe (Berri et al., 2007; Parisi et al., 2006). Q fever, similar to many other zoonotic diseases, has dual impacts on humans and animals, which makes it difficult to estimate the true burden of the disease in affected areas using a single standardised measure of global disease burden such as disability-adjusted-life year (DALY) (Grace et al., 2012). The DALY measure addresses human health, and can only partially estimate human disease burden because it does not capture medical costs of illness to individuals or the social costs of illness which may include costs of the acquisition of health care facilities and disease control and eradication programmes (Grace et al., 2012).

Since there is no available tool to estimate the overall burden of Q fever, the overall societal burden of the disease may be currently underestimated. The impact of the disease has mostly been recorded during outbreaks. The epidemic reported in the Netherlands between 2007-2010 was estimated to be 307 millions Euros (Van Asseldonk et al., 2013), overall cost to both human and livestock sectors. Q fever is also considered an occupational hazard, which means that farm workers, veterinarians and abattoir workers among others are at increased risk of infection, and could greatly impact on their livelihoods. Chronic fatigue syndrome (CFS) has been suggested as a major health problem in human infection, and significantly contributes to the disease burden (Van Asseldonk et al., 2013). It has been suggested that CFS can last for 5-10 years and affected individuals are unlikely to work full capacity, which could greatly impact on their welfare and wellbeing (Tempelman et al., 2011). Investigation into the financial impact of a Q fever outbreak in the Netherlands, demonstrated that 64% of all income related losses at both individual and organization levels were caused by chronic fatigue syndrome (Van Asseldonk et al., 2013). There are also other potential costs that can be incurred in the purchase of treatment materials at individual levels and government control programmes.

In livestock, the disease is associated with reproduction and performance failures such as abortion, stillbirth, weak neonates and infertility problems that greatly impact on production.

There are scant data on the impact of coxiellosis on livestock productivity in poor nations. Livestock production related losses could result in the loss of an income source that would likely provoke poverty in livestock owners in marginalised settings who may not have other sources of livelihood. Additional costs for veterinary services can also be incurred if used. The control of outbreaks of coxiellosis may require culling of chronically infected animals and closure of farms, which would have an enormous effect on farmers and government agencies if compensation is considered.

3.1.7 Aims and objectives

This study aims to describe the exposure patterns of *C. burnetii* infection among livestock populations in a range of agro-ecological settings in Tanzania. The prevalence across different ruminant livestock species and the potential effect of livestock related risk factors will be determined.

The key objectives are:

1. To describe and compare factors associated with *Coxiella* seropositivity status in cattle, sheep, and goats.
2. To identify determinants of *Coxiella burnetii* seropositivity among livestock species, that is, if the presence of another species of livestock in the same household or herd will be associated with the serostatus of others.

3.2 METHODOLOGY

3.2.1 Data source

All of the raw data for this study, including the ELISA results and linked risk factor data used for this chapter were sourced from the Bacterial Zoonoses project. This is a Biotechnology and Biological Sciences Research Council and the National Institute of Health (BBSRC-NIH) funded project on the impact, ecology and social determinants of bacterial zoonoses in northern Tanzania. Details of the field data collection and *C. burnetii* serological analyses are included here to provide a clear description of the sample collection and linked data available through the Bacterial Zoonoses project. These data were collected between July 2013 and November 2014.

3.2.2 Summary of field data collection

The detail of the field data collection processes has already been described (Chapter Two), and involved a cross-sectional survey of livestock-keeping households. The sampling strategy involved a multi-stage random selection process starting from the district to the final selection of households from where animals were identified. At each sampled household, data were gathered on livestock ownership (numbers of adult and juvenile cattle, sheep and goats present at each household).

Livestock Sampling

Random sampling was used to select livestock-keeping households (as described in Chapter Two). At each selected household up to 15 cattle, 15 sheep and 15 goats were sampled. For households with >15 cattle, sheep or goats, 15 individuals were selected and sampled. Selection of individuals was essentially opportunistic, but adult females were prioritized where feasible. At households with ≤ 15 livestock of a particular species, all individuals of that species present were sampled.

Venous blood samples were collected from all sampled livestock. In addition, data on the age, sex and breed of animals were collected. The ages of the animals were determined using the standard dentition criteria for livestock (Table 2).

Table 2: Age categories for livestock species sampled

Age Category	Description	Age in months	
		Cattle	Sheep & Goats
1_Temp	Temporary teeth only	<12	<12
2_2T	2 permanent teeth eruption	12-24	12-18
3_4T	4 permanent teeth eruption	24-36	18-24
4_6T	6 permanent teeth eruption	36-42	24-30
5_Full	Full permanent teeth	42-60	30-42
6_FullWorn	Full worn permanent teeth	>60	>42

Information on the vaccination history of all sampled animals was obtained at the time of sampling and no vaccination against coxiellosis was reported in any of the households. All blood samples were collected into 10mL red-top vacutainers for serum separation, centrifuged on the day of collection and serum separated, and refrigerated for up to 24 hours before transfer to storage in -80°C. Serum samples were heat treated (56°C for 2 hours) in Tanzania to inactivate any potential harmful agents, shipped to the laboratory at the University of Glasgow on dry ice and upon arrival to the laboratory were immediately transferred and stored in -80°C freezers.

3.2.3 Summary of Q fever ELISA procedure

ELISA testing was carried out by Dr Nick Wheelhouse, at the Moredun Research Institute. A commercial ELISA kit, LSIVet™ Ruminant Q Fever Serum/Milk ELISA Kit, marketed by Life Technologies Limited, was used for the serological analysis. The test is considered to have antibody detection sensitivity of 85% and specificity of 95% (Courcoul et al., 2010). Serum samples were screened for IgG antibodies to Phase I and II purified antigens from the reference Nine Mile strain of *Coxiella burnetii* (Guatteo et al., 2008). Using the pre-coated ELISA plate, 100µl of diluted sera (1:400 dilution) was added to the test well and incubated for 60 minutes at 37°C. The plate was washed after first incubation cycle, and 100µl of a peroxidase-labelled anti-ruminant immunoglobulin G (IgG) conjugate was then added and incubated for 60 minutes at 37°C. After the second incubation, the plate was washed and 100µl of TMB substrate was added to all wells. After 15 minutes incubation in a dark cupboard at room temperature, the reaction was stopped with a stop solution. Plates were read using an ELISA plate reader at 450nm single wave length. The results were expressed as

% OD which was calculated as follows:

$$\% \text{ OD} = [(\text{OD sample} - \text{OD negative control}) / (\text{OD positive control} - \text{OD negative control})] * 100.$$

Based on the recommendation of the manufacturer, livestock samples were considered positive if the % OD ≥ 40 and negative if %OD < 40 .

3.2.4 GIS maps of study sites

Complete details of the maps developed for the Bacterial Zoonoses project study sites have already been described (Chapter Two). In addition, separate maps were developed for each livestock species that was tested for antibodies against *C burnetii*. For example, there were maps showing the results for cattle, sheep and goats separately.

3.2.5 Statistical analysis

Before analysis, the data set was checked for errors (visualization and eyeballing) that may arise due to missing values, inconsistencies, inaccuracies and outliers. All statistical analyses were performed using the R programming language (R Development Core Team, 2014). The seroprevalence of exposure to *C. burnetii* in cattle, sheep and goats was determined.

Individual serostatus, with a binary outcome (ELISA positive or negative), was the outcome or dependent variable in all analyses. Generalized linear mixed models (GLMMs) (using the ‘lme4’ package in R) were used to determine whether there was an association between the dependent variable and host specific and environmental variables considered as independent variables (fixed effects). The fitted binomial family model with a logit link function included a random effect term to account for household level variation. P values < 0.05 were considered statistically significant.

The following variables (fixed effects) were screened during univariable analysis:

(A) Animal husbandry system (setting); a 3-level factor: Pastoral, Agropastoral or Peri-Urban.

(B) Age of animal; a 5-level factor: 1-Temp, 2_2T, 3_4T, 4_6T, 5_Full or 6_Fullworn (See Table 2 for details).

- (C) Sex; a 2-level factor: Male or Female.
- (D) Presence of cattle in the household; 2-level factor: Yes or No
- (E) Presence of sheep in the household; 2-level factor: Yes or No
- (F) Presence of goats in the household; 2-level factor: Yes or No
- (G) Presence of positive cattle in the household; 2-level factor: Yes or No
- (H) Presence of positive sheep in the household; 2-level factor: Yes or No
- (I) Presence of positive goats in the household; 2-level factor: Yes or No

The univariable models for the fixed effects ‘D’ to ‘I’ (presence of cattle, sheep, goats and presence of positive cattle, sheep, and goats) were only applicable for some households depending on the livestock species being modelled. For example, the effect of the presence of positive sheep or goats was assessed in models of cattle serostatus but the presence of cattle variable was not included in the model of cattle exposure (because cattle were present by definition at any household with results from sampled cattle).

Separate univariable model sets were constructed for each livestock species (cattle, sheep and goat) to evaluate factors associated with exposure in these different populations. This was followed by variable selection and then multivariable analysis for each species in turn. For the univariable analyses, likelihood ratio tests (LRT) were used to compare each univariable model with the null (intercept only) model that is nested. Variables with a p-value < 0.2 in the univariable LRT were selected for inclusion in the full multivariable model for each livestock species. Each full multivariable model was reduced by sequentially removing variables that had the highest (non-significant) p-value in LRTs comparing the current model to the set of models with one additional variable removed. This process was repeated until all variables remaining in the model had a significant p-value (<0.05) in the LRT (e.g. when the inclusion of all remaining variables significantly improved model fit). Errors in the models, for example failure to converge, were addressed using model optimizers such as the Bound Optimization By Quadratic Approximation (bobyqa) (Powell, 2009) that is compatible with the ‘New version of lme4’, while evidence of poor model fit resulting in large standard error values was resolved by combining variable levels where applicable.

The coefficient values in the final multivariable models and the relevant univariable models were compared to identify any problems of collinearity. Biologically plausible two-way interactions between main effects were also examined and significance was declared at $p < 0.05$.

3.3 RESULTS

3.3.1. Summary statistics

A total of 1436 ELISA results were available for the livestock population sampled. The majority of samples were from goats 45.8% (658/1436), the least from sheep 24.5% (352/1436) and the remainder from cattle 29.7% (426/1436). There were more female 71.2% (1023/1436) than male 28.8% (413/1436) animals sampled. More than half of the livestock samples, 58% (833/1436) were from the pastoral setting, 25% (359/1436) from peri-urban and 17% (244/1436) from agro-pastoral. More livestock were sampled from households where multiple livestock species were present than single species households (Table 3). The proportion of sheep sampled from households with at least one cattle present was 80.7% (284/352), with 44.9% (158/352) coming from a household where one or more ELISA positive cattle were present. Of the sheep samples, 86.4% (304/352) came from households where goats were present, with 72.7% (256/352) coming from households with one or more positive goats. Summary information for each species is shown in Table 3.

Table 3: Summary of the livestock populations sampled and *Coxiella* ELISA tested

Livestock Species	Variable	Variable sub-category	Number Sampled	% sampled
Sheep (n=352)				
	<i>Age</i>	1_Temp	78	22.2
		2_2T	46	13.1
		3_4T	41	11.7
		4_6T	21	6.0
		5_Full	146	41.5
		6_FullWorn	19	5.4
	<i>Setting</i>	Agro-pastoral	40	11.4
		Pastoral	272	77.2
		Peri-urban	40	11.4
	<i>Sex</i>	Male	101	28.7
		Female	251	71.3
	<i>Other species on farm</i>	Cattle present	284	80.7
		Cattle absent	68	19.3
		Goats present	304	86.4
		Goats absent	48	13.6
	<i>Other ELISA positive species on farm</i>	Cattle positive	158	55.6
		Cattle negative	126	44.4
		Goats positive	256	84.2
		Goats negative	48	15.8

Table 3: cont.

Livestock Specie	Variable	Variable sub-category	Number Sampled	% sampled
Cattle(n=426)	Age	1_Temp	153	35.9
		2_2T	26	6.2
		3_4T	34	8.0
		4_6T	14	3.3
		5_Full	168	39.4
		6_Fullworn	30	7.1
	Setting	Agro-pastoral	71	16.7
		Pastoral	296	69.5
		Peri-urban	59	13.8
	Sex	Male	112	26.3
		Female	314	73.7
	Other species on farm	Sheep present	266	63.4
		Sheep absent	160	37.6
		Goats present	288	67.7
		Goats absent	138	32.3
	Presence of ELISA positive onfarm	Sheep positive	245	92.1
		Sheep negative	21	7.9
Goats positive		240	83.3	
Goats negative		48	16.7	
Goats(n=658)	Age	1_Temp	183	27.8
		2_2T	62	9.4
		3_4T	65	9.9
		4_6T	61	9.3
		5_Full	231	35.1
		6_FullWorn	54	8.2
	Setting	Agropastoral	133	20.2
		Pastoral	265	40.3
		Peri-urban	260	39.5
	Sex	Male	458	69.6
		Female	200	30.4
	Other species on farm	Sheep present	337	51.2
		Sheep absent	321	48.8
		Cattle present	409	62.2
		Cattle absent	249	37.8
	Presence of ELISA positive onfarm	Sheep positive	265	78.6
		Sheep negative	72	21.4
Cattle positive		178	43.5	
Cattle negative		231	56.5	

Note: % sampled values for the variable 'Presence of ELISA positive on farm' is a further classification of the variable 'Other species on farm', 'sub-category Sheep, Cattle or Goats Present'. It represents households where sheep, cattle or goats present had tested either positive or negative.

3.3.2 Study sites for the Bacterial Zoonoses project

The study sites for the Bacterial Zoonoses project where livestock species were sampled are indicated in Figure 1 (cattle), Figure 2 (sheep), and Figure 3 (goats).

Figure 1: Map of study sites for the Bacterial Zoonoses project where cattle were sampled

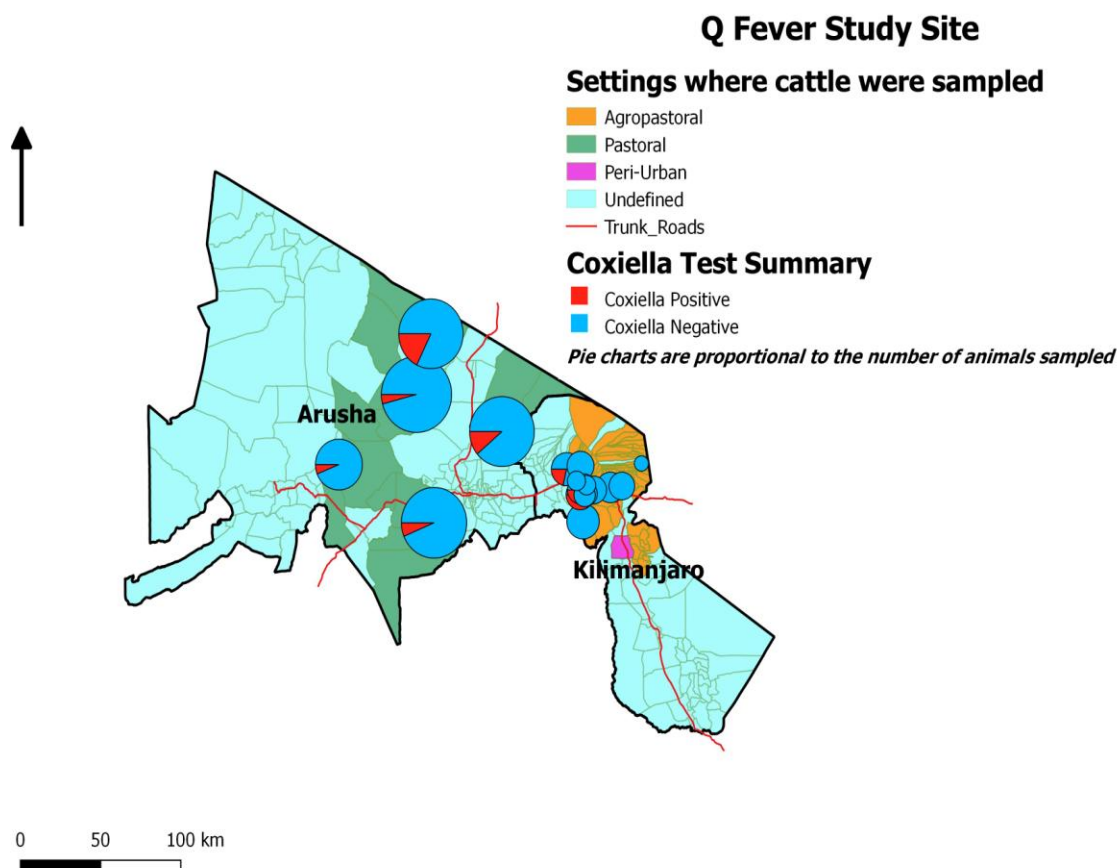


Figure 2: Map of study sites for the Bacterial Zoonoses project where sheep were sampled

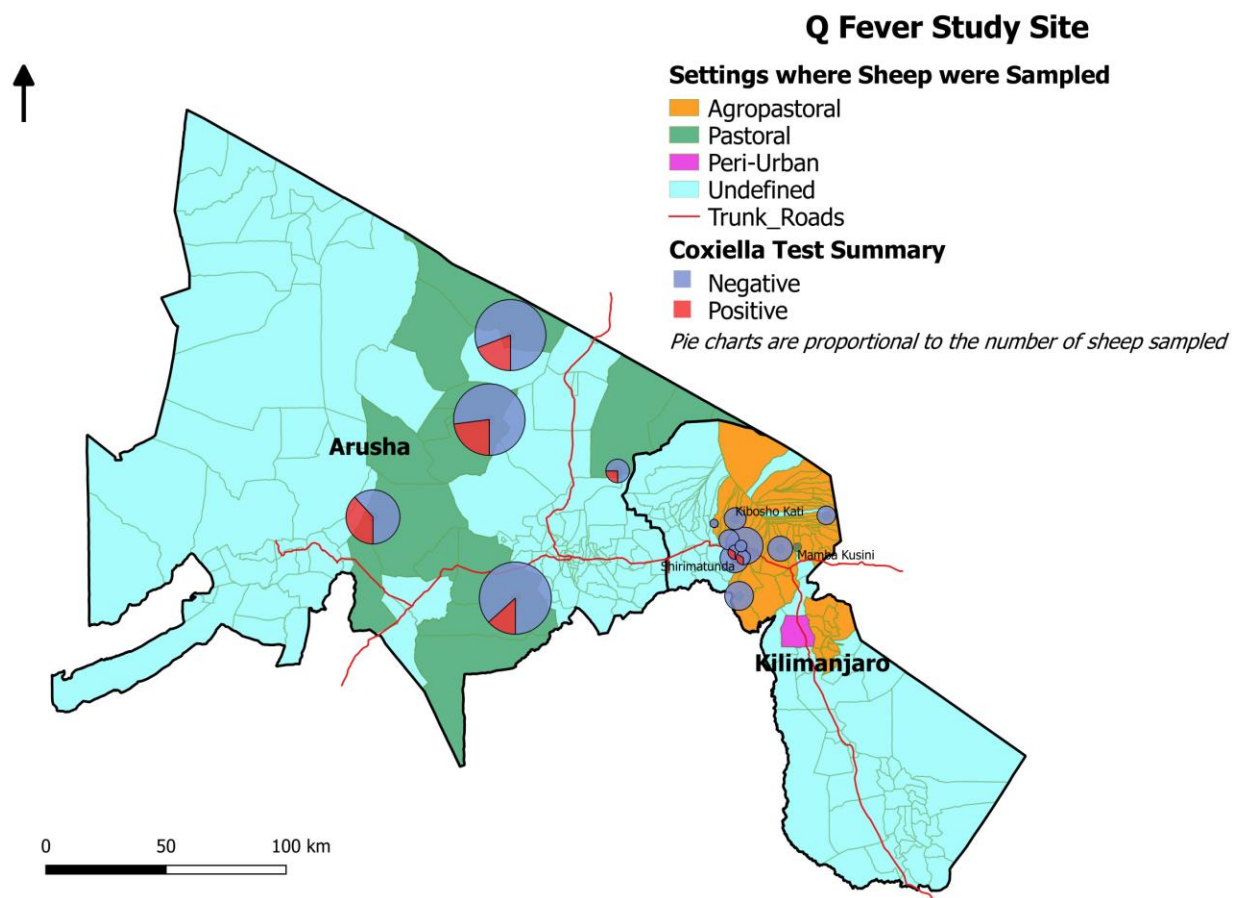
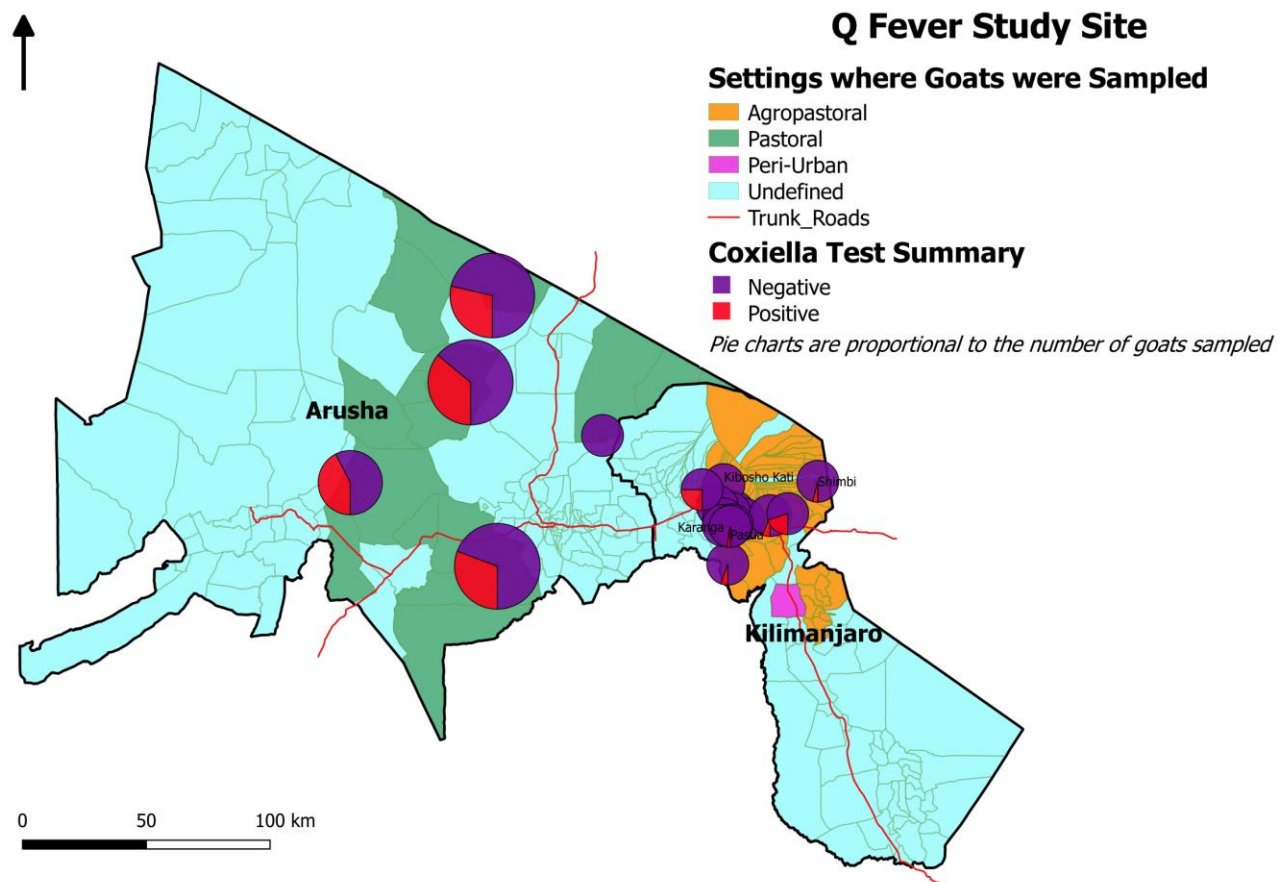


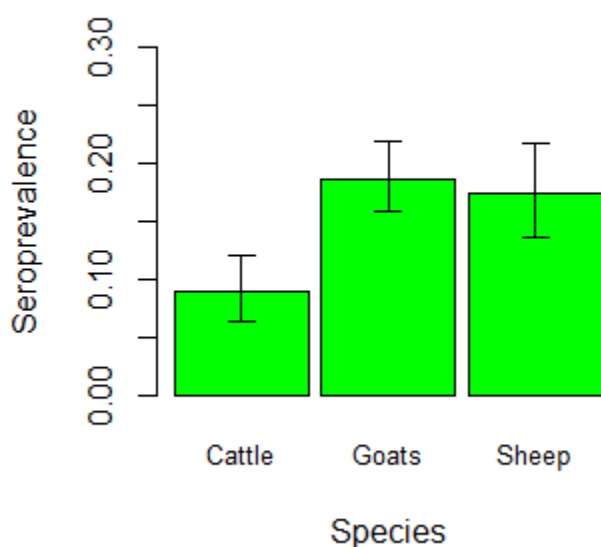
Figure 3: Map of study sites for the Bacteria Zoonoses project where goats were sampled



3.3.3 Seropositivity in livestock

The overall seropositivity in livestock was 15.5% (222/1436), with 8.9% (38/426) cattle, 17.3% (61/352) sheep, and 18.7% (123/658) in goats (Figure 4).

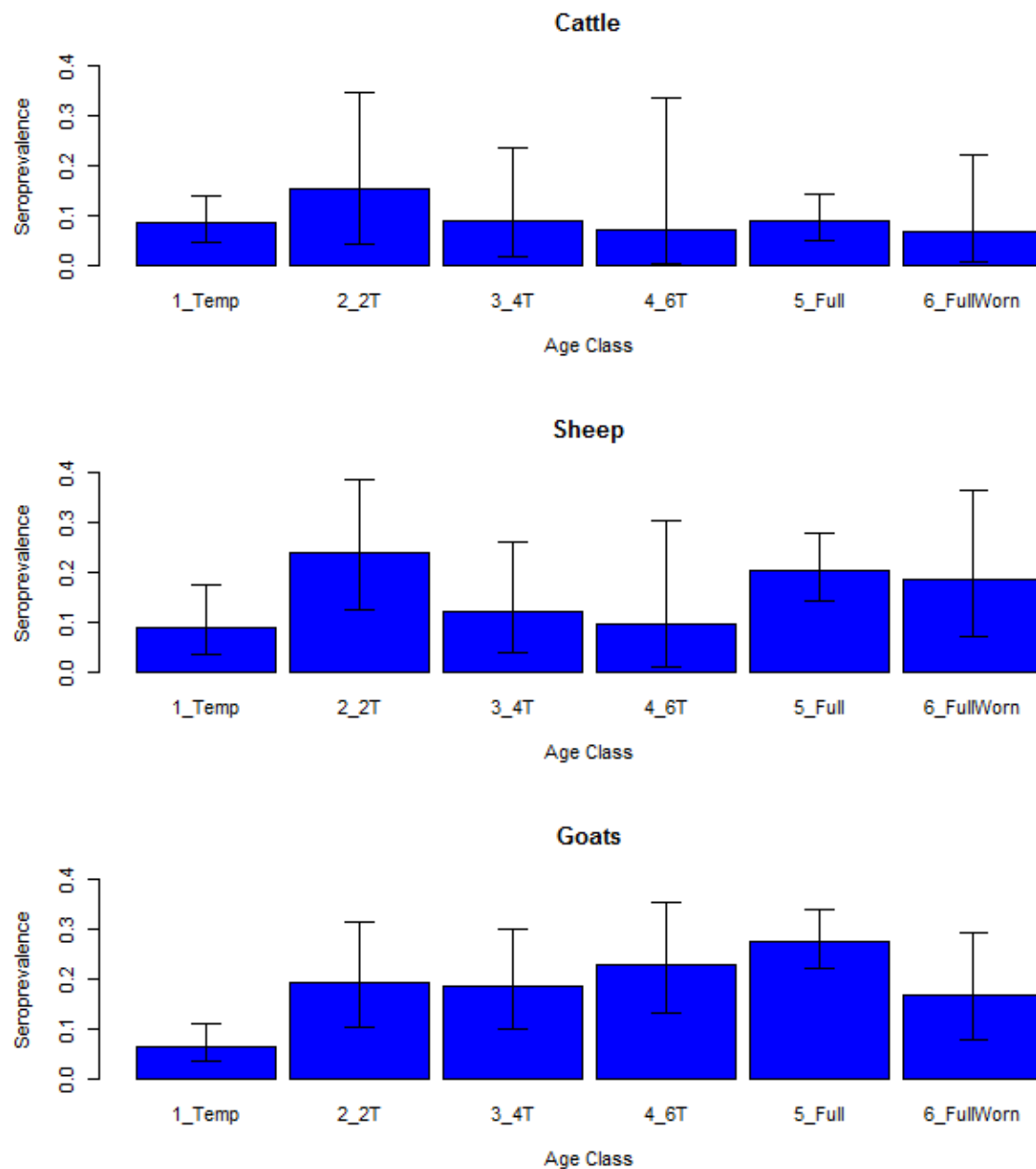
Figure 4: Figure showing seroprevalence across livestock species, vertical bars represent 95% confidence interval



Age

Seroprevalence was the same across age groups for cattle and sheep, but the pattern was different in goats (Figure 5). In goats, there was a gradual increase in the percentage seropositivity across age groups from 6.7% in the youngest age group (with temporary incisors estimated to be less than one year of age), which appears to peak at 27.7% in the adult age class (animals with a full mouth/complete set of permanent teeth estimated to be 4 years of age), and then a decrease in the oldest animal group (16.7%) with full worn teeth (estimated to be more than 4 years of age).

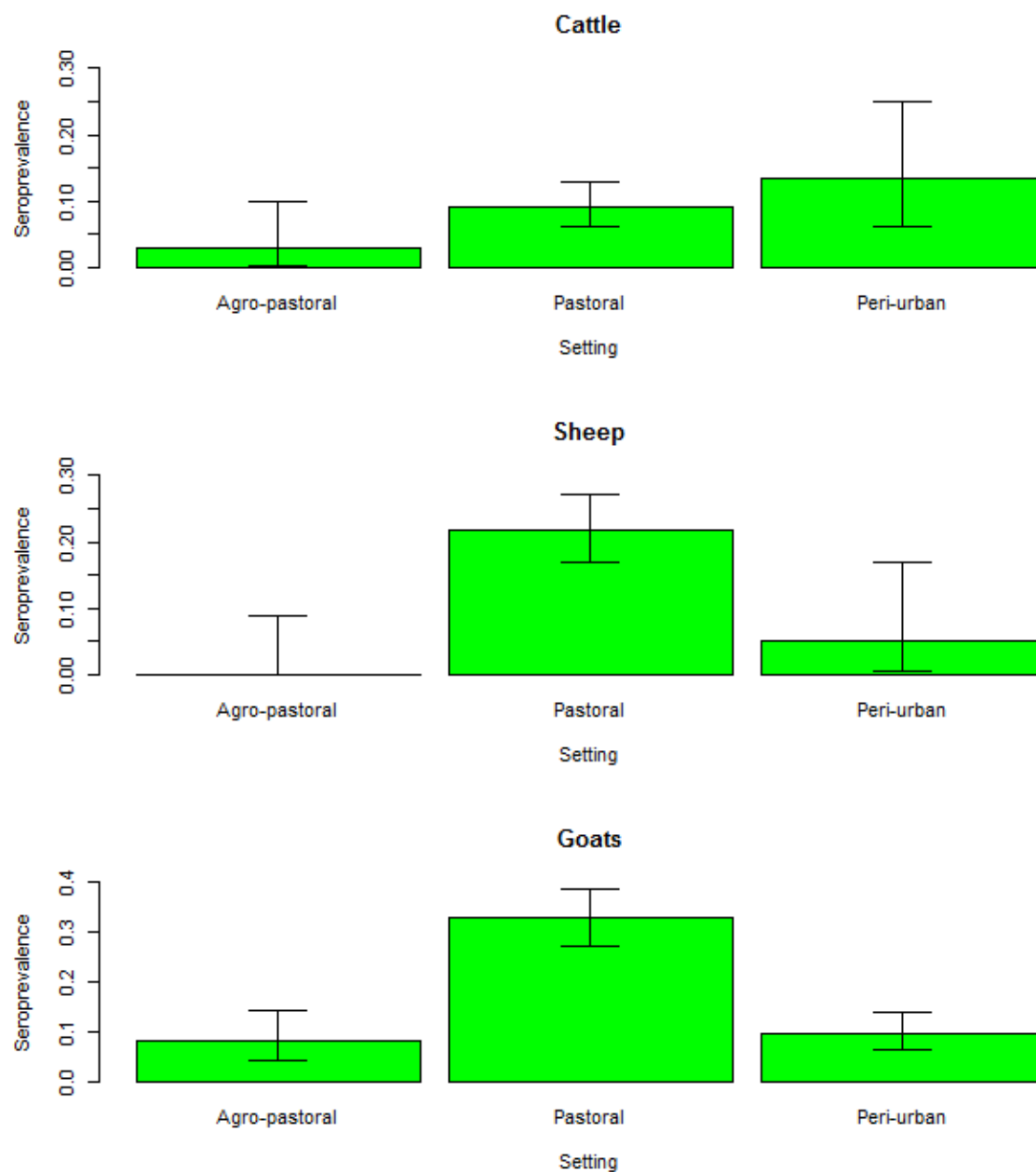
Figure 5: Figure showing seroprevalence across livestock species by age class as determined by dentition, vertical bars represent 95% confidence interval



Setting

The seroprevalence was the same across agro-ecological settings in cattle, but differs in sheep and goats (Figure 6). In sheep and goats, seroprevalence varies across agro-ecological settings, with the highest level observed in the pastoral systems compared to peri-urban and agropastoral settings. In addition, there was no positive sheep in the agropastoral setting (Figure 6).

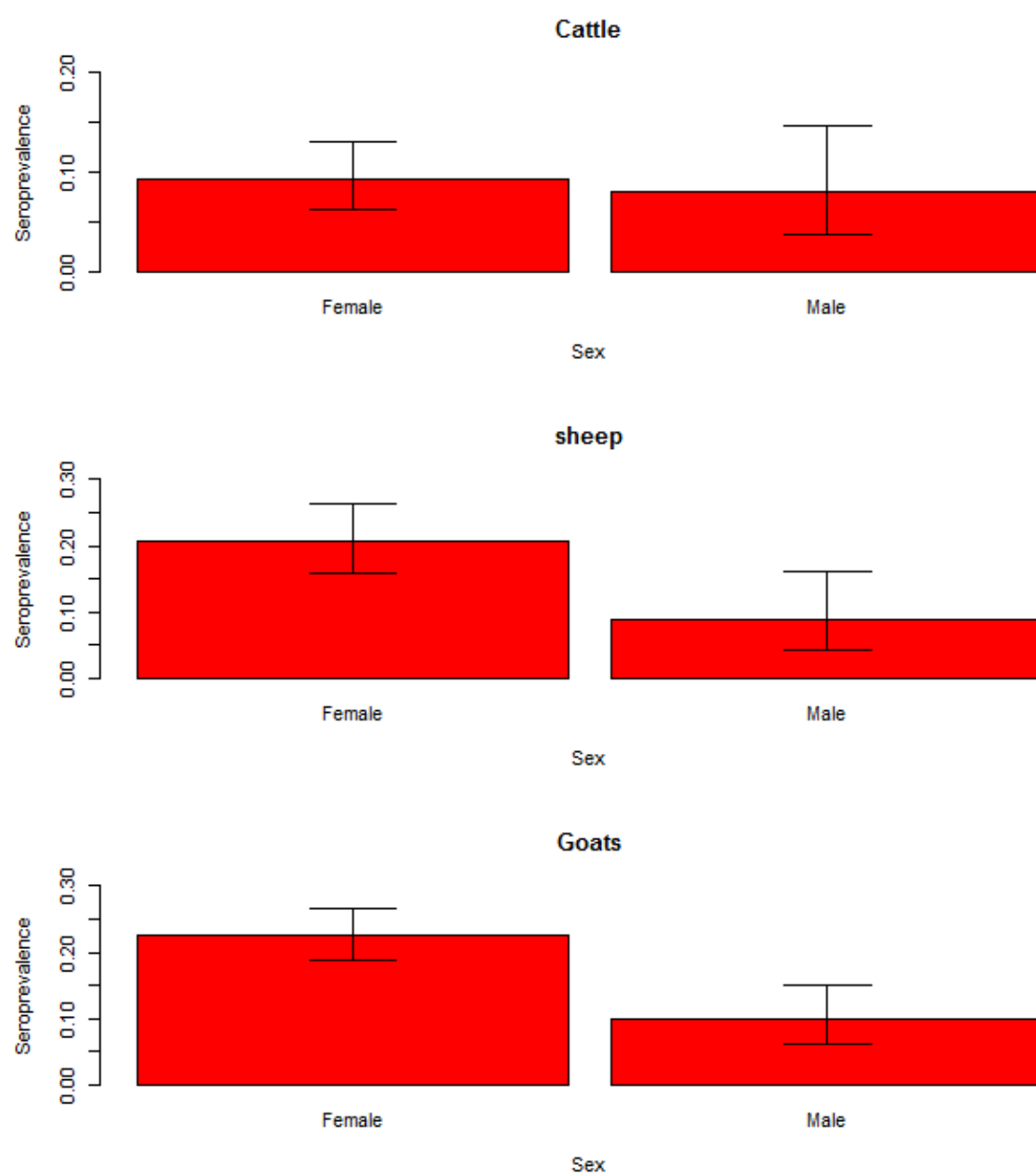
Figure 6: Figure showing seroprevalence across livestock species by animal management systems, vertical bars represent 95% confidence interval



Sex

There was no difference in seroprevalence between females and males in cattle, while seropositive was higher in female than male sheep and goats (Figure 7).

Figure 7: Figure showing seroprevalence across livestock species by sex, vertical bars represent 95% confidence interval



3.3.4 Univariable generalised linear mixed model (GLMM) analyses and likelihood tests (LRT)

GLMMs and LRT for cattle

The likelihood ratio tests of the univariable models indicated no evidence of significant associations between cattle serostatus and setting ($\chi^2=2.1$, $df=2$, $p=0.344$), cattle serostatus and age ($\chi^2=1.6$, $df=5$, $p=0.900$) or between cattle serostatus and sex ($\chi^2=0.2$, $df=1$, $p=0.652$). In addition, the LRT also shows that the presence of goat or sheep in the same household as cattle had no effect on cattle serostatus ($\chi^2=0.03$, $df=1$, $p=0.871$) and ($\chi^2=0.11$, $df=1$, $p=0.738$) respectively. Likewise, it was observed that the presence of at least one seropositive goat or sheep in same household as cattle does not affect the serostatus of cattle ($\chi^2=0.05$, $df=1$, $p=0.832$) and ($\chi^2=0.08$, $df=1$, $p=0.781$) respectively.

GLMMs and LRT for sheep

An error was observed for the coefficient estimates for the setting variable category ‘agropastoral’, which occurred because no sheep had tested positive in the region. Therefore, to allow robust estimation of the effect of this variable for other settings, test for any statistical significance and enable inclusion of the variable in the multivariable modelling the variable categories ‘agropastoral’ and ‘peri-urban’ were combined to form a new single factor level of ‘non-pastoral’. Thus, the setting variable that was used in the model of sheep data had two categories: pastoral and non-pastoral.

The results of the likelihood ratio tests of the univariable models showed significant associations between sheep serostatus and setting ($\chi^2=19.7$, $df=1$, $p=0.000$), and between sheep serostatus and sex ($\chi^2=6.8$, $df=1$, $p=0.009$). No evidence of a significant effect of age on sheep serostatus was observed ($\chi^2=10.5$, $df=5$, $p=0.06$).

There was no evidence to suggest that sheep are more or less likely to be seropositive if cattle are present in the farm ($\chi^2=3.8$, $df=1$, $p=0.05$), and also no effect of the presence of goats on the serostatus of sheep ($\chi^2=2.5$, $df=1$, $p=0.111$). However, the presence of at least one ELISA positive cattle or goat in the same farm as sheep, significantly increases the probability of sheep to test ($\chi^2=4.2$, $df=1$, $p=0.041$) and ($\chi^2=7.2$, $df=1$, $p=0.007$) respectively.

GLMMs and LRT for goats

The findings indicated significant associations between goat serostatus and setting ($\chi^2=7.2$, $df=1$, $p=0.007$), goat serostatus and age ($\chi^2=30.3$, $df=2$, $p<0.001$), and goat serostatus and sex ($\chi^2=17.4$, $df=1$, $p<0.001$).

It was also observed that goats are significantly more likely to test positive if cattle or sheep were present at the same farm, ($\chi^2=12.8$, $df=1$, $p<0.001$) and ($\chi^2=11.2$, $df=1$, $p<0.001$) respectively. Similarly, the presence of an ELISA positive cattle in the same farm as goats significantly affected the goats serostatus ($\chi^2=10.2$, $df=1$, $p<0.001$); and the effect of the presence of an ELISA positive sheep in the same farm as goat was highly significant on goat serostatus ($\chi^2=30.2$, $df=1$, $p<0.001$).

3.3.5 Multivariable generalized linear mixed models (GLMMs) analyses

Cattle

None of the variables considered were significantly ($p>0.2$ in the univariable LRTs) associated with cattle *Coxiella* ELISA status in this study, and multivariable analysis was not performed for cattle.

Sheep

The following variables: Setting, Age, Sex, Presence of Cattle, Presence of Goats, Presence of positive Cattle and Presence of positive Goats, were all included in the multivariable analysis. After adding all these variables to the model and the stepwise model selection process completed, only Setting (LRT, $p<0.001$), and Sex (LRT, $p=0.041$) remained in the final model. Before age was removed, the estimated parameters for all the levels were examined when included with Setting and Sex, and none of the levels [2_2T ($p=0.144$), 3_4T ($p=0.914$), 4_6T ($p=0.585$), 5_Full ($p=0.311$) and 6_FullWorn ($p=0.092$)] were significant. The removal of Age and other non-significant variables, improved the overall parameter estimates (such as standard error and coefficient) of the final model predictors (Table 4). The following was the order of removal of non-significant variables: Presence of positive Cattle, Presence of positive Goat, Presence of Cattle, Presence of Goat, and Age.

The interaction of Sex and Age was also examined, but this was not feasible to model robustly with the sheep data due to insufficient data at different age categories, and LRT indicated that the model without the interaction term was a better fit ($p=0.05$). There was no evidence of collinearity observed in the final model for sheep.

The final model (Table 4) shows that sheep in the pastoral regions are significantly more likely to test positive compared to those in non-pastoral regions ($p=0.001$), OR=10.7, 95% CI (6.5-14.9). Female sheep were significantly more likely to test positive compared to males ($p=0.012$), OR=2.6, 95% CI (1.2-5.6).

Table 4: Summary of the final model for Sheep

Variable	Category	Estimate	SE	z-value	p-value*	OR (95%CI)
Setting						
	Non-pastoral	-	-	-	-	-
	Pastoral	2.367	0.733	3.230	0.001	10.7 (6.5-14.9)
Sex						
	Female	-	-	-	-	-
	Male	-0.963	0.388	-2.480	0.012	2.6 (1.2-5.6)

**P-value indicates the level of significant difference for each category when compared to the reference group for the variable; SE-standard error; OR-odd ratio; CI-confidence interval.*

Goats

The main effects included in the multivariable modelling for goat serostatus were Setting, Age, Sex, Presence of Cattle, Presence of Sheep, Presence of positive Cattle and Presence of positive Sheep. After adding all of these variables to the model, it failed to converge due to errors of redundant variables, collinearity and variable level combinations where no goat was ELISA positive. The variable Presence of positive sheep was dropped from the model due to redundancy, and LRT (LRT, $p=0.889$) supported the removal of the variable. Other non-significant variables were removed in a step-wise order starting with the variable with highest P-value. The following was the order of removal of non-significant variables: Presence of positive Cattle, Presence of positive Sheep, Presence of Cattle, and Presence of Sheep. The final model included Setting (LRT, $p=0.001$), Age (LRT, $p=0.027$), and Sex (LRT, $p=0.027$).

To allow robust estimation of an interaction between Age and Sex, the age groups 12-18 months and 18-24 months were combined to form a new group 12-24 months because there was no male positive goats in the age group 18-24 months and large standard error were observed, which prevented model convergence. There was a significant interaction between

Age and Sex (Table 5), and LRT indicated that the final model with the interaction terms was a better fit ($p=0.005$). There was no evidence of collinearity observed in the final model for goats.

The final model (Table 5) shows that goats in the pastoral settings were more likely to be ELISA positive compared to agropastoral ($p<0.001$), OR=5.8, 95% CI (3.2-8.3), while there was no evidence to suggest goats in the peri-urban settings were significantly more or less likely to test positive compared to agropastoral ($p=0.798$), OR=1.1, 95% CI (0.43-3.0). Only adult goats of about 3-4 years of age with full developed permanent teeth were significantly more like to test positive compared to the reference group, which consists of young goats less than 12 months of age with temporary teeth ($p=0.044$), OR=2.7, 95% CI (1.1-6.9) (Table 5). Effects of interaction further suggest that goats in the age group 2-2.5 years and oldest animals of more than 4 years are more likely to be seropositive compared to the reference group, but this depends on whether they are male or female. For example, adult male goats were significantly more likely to test positive than female goats of the same age group, while there was no evidence of significant effects of interaction in other age groups (Table 5). Male goats were significantly less likely to test positive compared to females ($p=0.042$), OR=3.8, 95% CI (1.1-13.7), but this also depends on the age class of the male goats (Table 5).

Table 5: Summary of the final model for Goats

Variable	Category	Estimate	SE	z-value	p-value*	OR (95%CI)
Age						
	1_Temp	-	-	-	-	-
	2_2T&3_4T	0.877	0.543	1.613	0.107	2.4 (0.8-7.0)
	4_6T	0.193	0.635	0.304	0.761	1.2 (0.4-4.2)
	5_Full	0.980	0.486	2.016	0.044	2.7 (1.1-6.9)
	6_FullWorn	0.683	0.645	1.057	0.290	2.0 (0.6-7.0)
Sex						
	Female	-	-	-	-	-
	Male	-1.332	0.655	-2.036	0.042	3.8(1.1-13.7)
Age*Sex						
	2_2T&3_4T:Male	0.221	0.914	0.242	0.809	1.3 (0.3-7.5)
	4_6T:Male	2.975	1.056	2.817	0.005	19.6 (11.7-27.5)
	5_Full:Male	-0.038	0.947	-0.040	0.968	1.0 (0.2-6.7)
	6_FullWorn:Male	3.156	1.462	2.158	0.039	23.5 (15.0-31.9)
Setting						
	Agro-pastoral	-	-	-	-	-
	Peri-urban	-0.127	0.495	-0.256	0.798	1.1 (0.4-3.0)
	Pastoral	1.749	0.477	3.671	0.000	5.8 (3.2-8.3)

*P-value indicates the level of significant difference for each category when compared to the reference group for the variable; SE-standard error; OR-odd ratio; CI-confidence interval

3.4 DISCUSSION

The findings of this study demonstrate widespread exposure to *C. burnetii* in cattle, sheep and goats, in northern Tanzania, but reported a variation on the infection prevalence across livestock species and animal management systems. Livestock related risk factors differed between species, with some identified in sheep and goats, but none in cattle. Seroprevalence was highest in goats (18.7%), followed by sheep (17.3%) and the least in cattle (8.9%). These observations may indicate genuine variation in prevalence across livestock species due to different patterns and level of exposures, but with some similarities between sheep and goats compared to cattle. The difference in sub-types of *Coxiella* within livestock species, could also partly explain these observations. The results also indicated that presence of other species in the farm whether they have been exposed or not did not seem to have any effect on the seroprevalence in goats or sheep or cattle. It is possible that at herd or farm level, animals already share the same risk factors for exposure that may exert a bigger effect than detecting a positive or negative animal in the herd.

Evidence from recent reviews on animal coxiellosis in Africa, did not report any reliable studies that had investigated risk factors for *C. burnetii* infection in livestock populations in Tanzania (Njeru et al., 2016; Vanderburg et al., 2014). Therefore, there are limitations in comparing this study with other relevant studies within the region because it is probably the first ever classical sero-survey on the prevalence of livestock coxiellosis conducted in Tanzania. However, a study with similar design conducted in neighbouring Kenya, reported seroprevalence to be highest in goats compared to other livestock, which is consistent with this study (Knobel et al., 2013). The study in Kenya reported overall seroprevalence values that were higher (except in sheep) compared to our study (cattle=28%, sheep =18% and goats =32.0%). In addition, an ELISA serological survey of cattle based on banked sera collected in the year 2000 from Cameroon, Central Africa, reported prevalence of 32% (Scolamacchia et al., 2010). This comparison may show that *C. burnetii* infection in livestock differs across regions but the differences seen may also be due to the period when the studies were conducted, different ELISA kits and sampling methods. In small ruminants, seroprevalence also differs from other studies. In goats, a recent review of livestock seroprevalence reported the highest prevalence in Sudan (24%) and Egypt (23%), with the least in Chad (13%) among studies reviewed (Vanderburg et al., 2014). A similar pattern was also reported in sheep, where seroprevalence was highest in Egypt (33%) and least in Chad (11%) (Vanderburg et al., 2014). The observation in our study showed lower infection levels in both sheep and goats,

compared to these other relevant studies. These studies also differed though in the period when study was conducted and sampling methods, which could all account for the dissimilarities.

Coxiella burnetii infection levels in cattle were different compared to sheep and goats, with none of the risk factors investigated having any effects on cattle *Coxiella* serostatus. The shedding characteristics of *C. burnetii* in cattle may partly explain reasons for the observed seroprevalence because shedding appears to be the most important source of exposure through environmental contamination (Guatteo et al., 2006). In cattle, shedding is almost entirely in milk, and in small quantity (Rodolakis et al., 2007), indicating less environmental contamination compared to sheep and goats where large quantity of the bacteria is shed in all routes (Berri et al., 2007, 2001). Even though environmental contamination may be less in cattle, the long duration of *C. burnetii* shedding in milk, and the ability of the pathogen to persists in areas of good farm hygiene and transmission during milking process, could explain the higher infection levels observed in intensively managed dairy cattle in the peri-urban systems compared to other agro-ecological settings.

There was no clear explanation on why no risk factors were identified for cattle in the analysis. A possible reason could be that the dataset for cattle was not sufficiently powered to identify a significant effect when one actually exists because of relatively low prevalence of the disease in cattle in the region. Alternatively, it is also possible that the livestock related risk factors assessed are actually not very important determinants of cattle sero status. Since inhalation is the major route of *C. burnetii* infection in cattle, environmental factors, such as wind speed, low rainfall and extreme dryness, which were not directly measured in this study, may be more relevant. However, further studies would be required to evaluate these factors.

The highest seroprevalence in goats among other livestock species was expected based on evidence in literature that reported highest prevalence of livestock coxiellosis in goats (Knobel et al., 2013). Goats are considered to shed large quantity of *C. burnetii* through all routes (Arricau-Bouvery et al., 2003), thereby increasing environmental contamination and chances of exposure to other susceptible animals within infective distance. However, this was not investigated in the study. Several studies have also implicated goats as the major source of human infection due to their shedding characteristics (Guatteo et al., 2006). The high seroprevalence in goat may indicate potential risks to human, and when they are seronegative because shedding could still occur (Berri et al., 2007).

The result of this study shows that animal husbandry system is the main risk factor for caprine coxiellosis, with higher infection risks in pastoral system. System of animal husbandry has long been considered to affect several livestock diseases in Tanzania. It was identified as the major risk factor for bovine leptospirosis (Chapter Two) and other previous studies on leptospirosis (Assenga et al., 2015), foot-and-mouth disease (Lembo et al., 2012), have reported similar findings where animals in the pastoral systems of husbandry had significant higher disease risks than other agro-ecological settings. The demonstration of highest seroprevalence of caprine coxiellosis in the pastoral system is consistent with findings from other studies in this region, where pastoral systems were also identified as significant risk factor for higher levels of infection with *Mycobacterium bovis* (Cleaveland et al., 2005). As explained earlier (Chapter Two), there are certain characteristics of the pastoral systems of livestock farming that appears to drive high levels of infection. Features such as large herd sizes, wide-ranging grazing systems, high levels of mixing with animals from other herds, as well as contact with other types of small ruminants, potential exposure to wildlife populations, and generally low levels of bio-security have been described to increase exposure options.

The shedding of *C. burnetii* that is very common in goats could also account for the rapid infection spread in the pastoral systems. The low level of bio-security in the pastoral system means large quantity of the pathogen that is being expelled in birthing materials during kidding can potentially spread within farms much quicker in large herd size than small ones. Large herd size has already been implicated in several infections disease (Chapter Two) because there are more chances of infected animal being present and increased infection transmission to others. *Coxiella burnetii* can also survive for several months in the soil (Drew, 2004), and transmission can occur to other migratory herds typical of the pastoralist management system when they graze on contaminated pasture. The risks can even become higher if the pathogen is aerosolised as dust particles and can spread across several farms during favourable seasons and environmental conditions. Sexual transmission of *C. burnetii* has also been reported, which may be more critical in pastoralist systems where livestock keeping is mainly extensive with little or no control over animal breeding, thereby increasing the potential role of sexual transmission.

Arthropod transmission of *C. burnetii* can also be associated with pastoral systems where potential exists for the maintenance of their population due to large herd size, increased contacts with other domestic and wild animals. The pastoral systems can create the right ecological balance required for arthropod proliferation and increase agricultural factors that encourage contacts between arthropods and livestock (Stoker and Marmion, 1955). However, it is not clear how important tick transmission could be in the epidemiology of caprine coxiellosis (Babudieri, 1959).

The evidence in this study shows that seroprevalence in female goats (22.5%) was more than twice higher than males (10.0%). Evidence from experimental and epidemiological data better explains the reason for the observed difference in the serostatus of male and female goats. The uterus and mammary glands are the primary sites of chronic *Coxiella burnetii* infection in goats and cattle (Babudieri, 1959), and long term infection are mostly seen in female (dairy) animals (To et al., 1998). The pathogen localizes in these tissues and can persist for several years after initial infection, from where the bacterium is continuously shed in the milk and birthing tissues during lactation and parturition (Arricau-Bouvery et al., 2003). This chronic state of infection observed in female animals suggests antibodies against *C. burnetii* may last longer and diagnostic titre can be detected for longer period in females, which could also explain reasons for a higher seroprevalence.

The variation of seroprevalence across age groups observed in this study was similar to that reported in livestock in Cameroon (Scolamacchia et al., 2010), where it gradually increased with age and appears to peaked at the oldest animal age group. Livestock coxiellosis is prevalent in Tanzania, and one of the characteristics of an endemic infection, is that probability of becoming exposed will increase with age. Chronic infection of *C. burnetii* where antibody titres can persists for several months have often been reported (Arricau-Bouvery et al., 2003; Berri et al., 2001), indicating that the older animals have higher chances of testing positive. The probability of identifying a seropositive animal in older age groups would still be high even when antibody titre may wane, because repeated exposure of *Coxiella burnetii* is likely to be common in an endemic area. The pathogen possess well adapted features that enable it to be free living in the environment where it forms an infectious spore-like form that can survive for several months (Drew, 2004; McCaul, 1991). These survival properties of the pathogen in an endemic setting allows repeated cycles of exposure as animals get older. Sexual transmission is another possibility for the variation across age groups because exposure levels are more likely to increase with animals reaching

sexual maturity. However, sequestration of infection may occur as animal grow older, which could potentially explain the decrease in seropositivity in oldest animals (Figure 7).

The pattern of age-associated risk differed in male and female goats. Risk of infection was significantly higher in older males and in young females. It is expected that older female animals would be kept longer than male due to their reproduction potential and probably higher risks of exposure. However, an exception to this could be in adult male goats of reproductive age that are used for breeding. This category of animals may have more chances of repeated cycles of exposure than female animals because, although they are kept longer, they also have additional contacts (potential multiple exposure sources) when used for breeding with animals from other herds or distance farms. Currently in Africa, there are no standard measures of assessing animals for breeding soundness (including infectious diseases), and it is common practice for farmers to lend or rent their prized male animals to other farmers or herd for breeding, which could increase exposure levels in this animal category.

Similar to goats, husbandry system and sex were identified as significant risk factors for ovine coxiellosis, which is expected because sheep are likely to have shared livestock management characteristics to goats. In addition, shedding characteristics of sheep are similar to goats (Arricau-Bouvery et al., 2003), and persistence of *C. burnetii* in mammary glands is also observed in sheep, but less commonly than in goats. Notwithstanding the similarities in the livestock-keeping of small ruminants, age was not significant in sheep whereas it was highly significant in goats. This is surprising because in an endemic setting exposure is likely to increase with age. Another difference in the results for the multivariable models for sheep and goats, however, was that there was no significant evidence of an interaction between sex and age in sheep. The observed effect of sex was not dependent on age. It is possible that the practice of lending mature male animals for breeding may not be wide spread in sheep.

In conclusion, livestock coxiellosis is prevalent in Tanzania. The factors that are associated with the seroprevalence of *C. burnetii* seem to be different across livestock species and animal management and production related factors appears to be the most important. The widespread infection of *C. burnetii* across livestock species observed in this study poses a huge public health problem because sheep, goats and cattle are widely considered the most important reservoirs of human infection (Babudieri, 1959; Maurin and Raoult, 2010). It is likely that the prevalence of livestock coxiellosis will be associated with high incidence of

reproductive failure in affected herds leading to potentially high levels of exposure of other animals, livestock owners and their household members.

GENERAL DISCUSSION

The poor recognition of endemic zoonoses as a public health problem has often resulted in their under-reporting and underestimation of their impacts (Halliday et al., 2015; Maudlin et al., 2009). In addition, poor quality of data on the impact of zoonoses, especially those with low mortality in humans and animals, means they are likely to be overlooked by governments when prioritizing disease control in countries with limited resources. The consequence of this, however, has been the high prevalence of endemic zoonoses with significant disease burden on the most marginalized communities in poor countries where their impact is mostly felt. This thesis utilized the opportunity of a review of secondary literature, in the first chapter, to explore geographical patterns, and the frequency of reporting animal-related risk factors from publications on zoonotic pathogens that cause human fever across malaria endemic countries. Two of the 10 most common zoonotic pathogens that cause fever identified in the review, were investigated in detail (Chapter Two and Three) to identify the risk factors and determine exposure patterns for infection spread in livestock, and the implications for human transmission. In the following sections, results from each chapter were synthesized to describe the impacts and the wider implications of the high prevalence of endemic zoonotic diseases, and recommendations for disease control in a traditional endemic setting.

The key findings from this thesis indicate that zoonotic pathogens that contribute to human febrile illness are widespread across malaria endemic countries in both human and animal populations. However, it was observed that the frequency of reporting of the pathogens that cause human fever seems to be higher in regions where potential for outbreaks exist (Chapter One). International organizations such as the Global Leptospirosis Environmental Action Network (GLEAN) (Durski et al., 2014), which was established to specifically improve global and local interventions strategies in leptospirosis outbreaks situations, are most likely to focus attention in these regions where outbreaks are often reported. Research activities and publications relating to the pathogen are also likely to be clustered in these regions. Therefore, resulting in the under reporting of the pathogens in regions where outbreaks are less reported, but infection prevalence may still be high (Chapter Two and Three). In addition, other relevant organization such as World Health Organization (WHO) Global Burden of Disease (GBD) project that measures the overall burden of diseases, and which may be sourcing their information from GLEAN, could also underestimate the actual burden of the endemic zoonoses in areas of high endemicity, but less frequent outbreaks. The wider implications of

this, however, is that governments from countries where these diseases are endemic may be misinformed when relying on information from these organizations in prioritizing diseases to eradicate, resulting in neglect of endemic zoonoses.

The seroprevalence studies indicated that *Leptospira* spp. (Chapter Two) and *Coxiella burnetii* (Chapter Three), which had already been identified as frequently reported causes of human fever (Chapter One), are widespread in livestock. Evidence from epidemiologically linked livestock and human population studies in East Africa suggests that these pathogens co-circulate in human and animal populations in endemic settings (Assenga et al., 2015; Knobel et al., 2013), and the observations from this thesis (Chapter One), also show that these two pathogens concurrently infect human population. Relating this evidence to our current study, would suggest that animal-related risk factors would be important in zoonotic pathogen transmission, and the demonstration of widespread infection of *C. burnetii* and *Leptospira* serovar Hardjo in animals could pose a significant risk to humans. However, animal-related risk factors among other risk factors were often not considered and appreciated (Chapter One), indicating that preventive measures targeted at animal sources (reservoirs) are not being fully integrated.

The findings of high infection levels of *Leptospira* serovar Hardjo and *C. burnetii* in livestock population (Chapter Two and Three), and the identification of several incidents of concurrent infection in human febrile population (Chapter One), represent a significant problem for veterinary and a public health perspective. For example, livestock reproduction and performance problems that are associated with both pathogens exert a significant impact on livelihood. Direct human risks also exist, which is heightened by cultural practices such as drinking of raw milk. Even though this mode of human infection is considered rare in developed countries, because of the culture of milk pasteurization, it can be a significant infection source where raw milk consumption is widely practiced (Fishbein and Raoult, 1992). In pastoral livestock keeping communities where high levels of infections were detected (Chapter Two and Three), consumption of unpasteurised milk is common, and the implication of transmission of milk-borne zoonoses such as Q fever is heightened. Leptospirosis and Q fever in humans are regarded as occupational hazards where veterinarians, other livestock related occupations and abattoir workers are at great risk. Implications of the identification of high infection levels of *Leptospira* serovar Hardjo in the abattoir have been well described (Chapter Two). While direct contact with infective urine is considered the most important route of human exposure to *Leptospira* spp., transmission of *C.*

burnetii in humans is mainly by inhalation, which has been mostly implicated in epidemics (Hoek et al., 2010; Roest et al., 2011).

The persistence of nature of *Leptospira spp.* and *C. burnetii* in the environment means they can survive in farm waste materials that are routinely used as manure (Adler, 2001; Rodolakis et al., 2007). In areas of poor farm hygiene, and where animal waste materials are not treated before being used as farm manure or discarded, the risk of human infection through environmental contamination could be very high. For example, sheep manure was largely implicated in outbreaks of Q fever in Hungary (Gyuranecz et al., 2015), while spreading of goat manure was correlated with outbreak in humans in the Netherlands (Delsing and Kullberg, 2008). This suggests that communities in Tanzania, especially the agro-pastoralists that engage in mixed livestock and crop farming, would have higher risks of exposure if animal waste materials are used for crop production.

The widespread infection of *Leptospira spp.* and *C. burnetii* in livestock would suggest that human exposure will also be highly prevalent, especially in livestock-keeping communities where humans live in close contact with their animals, but this does not appear to be the situation based on current reviews (e.g. for Q fever) (Njeru et al., 2016). This may indicate poor recognition and under-reporting of the disease in humans and animals in these settings. It is clear that further studies are needed to investigate the prevalence of infection in linked livestock and human populations, and to evaluate the burden in both human and animal sectors caused by these important zoonoses.

One of the most noticeable finding in this thesis was the identification of no significant effect of mixed livestock keeping as a risk factor for coxiellosis (Chapter Three). Mixed-livestock keeping has long been considered a risk factor for several infectious diseases such as bovine tuberculosis (Tschopp et al., 2009), and FMD (Green et al., 2006; Kiss et al., 2006) where infection can jump from one livestock species network to another. However, this appears not to be important for *C. burnetii* as observed in this study where the presence of other species did not seem to affect the serostatus of other individuals. While age and cattle husbandry systems were significant determinants of bovine leptospirosis (Chapter Two), they were not important for bovine coxiellosis (Chapter Three), including other risk factors that were examined, suggesting potential difference in risk factors of these diseases. It is possible that the differences in the major transmission routes would have accounted for the observations. The main exposure route for *Leptospira* serovar Hardjo is by direct contact with infective

materials where proximity to infection source is critical and will increase chances of infection as observed in large herd size typical of the pastoral systems (Chapter Two). On the other hand, inhalation is the major route of *C. burnetii* infection in cattle, which indicates that exposure levels may depend on environmental factors that would facilitate spread such as wind speed, low rainfall and extreme dryness (Marrie, 1990b). The unidentified risk factors for cattle *Coxiella* serostatus also strongly suggest that robust studies would be required to assess livestock, environmental and ecological related risk factors for the disease, simultaneously.

General Recommendations

Immediate control of these endemic zoonotic diseases is challenging due to the complex epidemiology and the poor awareness of the disease for the population at risk, and challenges in the capacity to diagnose the disease both in animals and humans in northern Tanzania. We therefore have made the following recommendations, based on findings of this thesis, towards reducing the burden of endemic zoonoses:

- Community participatory workshops and sensitization programmes in the high-risk population such as farmers and their workers, abattoir workers, livestock market operators etc, to educate them on the presence of these zoonoses, sources of infection and their impact on human health and animals, are highly required.
- Controlling the disease in humans will be better achieved if it is risk-group targeted. For example educating livestock workers especially those in the abattoir about the risks of these diseases and wearing personal protective equipment (PPE) before handling animals will be crucial in reducing the disease in this target group. To further support this, government should be encouraged to initiate and enforce policies that prohibit animal contacts, especially in the abattoirs, without PPE.
- Raw milk should be adequately boiled before consumption.
- Birth tissues should be adequately disposed, especially those from sheep and goats.
- Livestock production systems are the major drivers of these diseases; improving and encouraging vaccination and treatment through partnership with the government (using ward level livestock officers) will help the farmers to uptake vaccination and treatment programmes.
- Education of farmers about basic ways these diseases can be controlled through good farm practice would be very effective. For example, the practice of recognising sick

animals and separating them from healthy ones using quarantine, early reporting of clinical signs to local vets, improved farm biosecurity such disinfecting fomites and human movement restrictions within farms; and wearing protective clothing when handling cattle and washing of hands with soap if in contact with animal before handling another, should be encouraged.

- Improving diagnostic capacity for these pathogens would be essential in reducing misdiagnosis, improve early diagnosis and implement the correct treatment plan to both animals and humans.

However, it is worthy of note that currently, some of these recommendations such as initiating new government policies and human behavioural change (e.g. washing of hands, wearing PPE and boiling of milk) may be difficult to implement at individual level in a typical endemic setting because of the challenges in changing human perception of disease risks. Encouraging good farm hygiene practice such as adequate disposal of birthing tissues and quarantine sick animals, however effective, is also a difficult process to enforce at farm level, but initiating these practices still remain a viable option for disease control. The problems of limited resources typical of these endemic countries could also prevent the government from acquiring laboratory diagnostic equipments and subsidising vaccination and treatment of sick animals and strengthening the veterinary services. Introducing good farm hygiene and management practice such as breeding control or disinfecting fomites in the pastoral communities may be very challenging. There is also the problem of justifying the economic sense in providing services such as veterinary care (e.g. diagnosis, quarantine, treatment and dip) in impoverished communities where livestock-keepers cannot afford the costs.

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Appendix A

List of Journals where abstracts were published

Medical Related Journals

Acta medica Indonesiana
 Acta Medica Iranica
 American Society of Tropical Medicine and Hygiene
 Annals of tropical medicine and Parasitology
 Annals of Tropical Medicine and Public Health
 Archives of Internal Medicine
 Asian Pacific Journal of Tropical Medicine
 British Medical Journal
 Chinese Medical Journal
 Ethiopian medical journal
 Indian Journal of Medical Microbiology
 Indian Journal of Medical Research
 Indian Journal of Medical Sciences
 Indian Journal of Ophthalmology
 International journal of clinical practice
 International Journal of Preventive Medicine
 Investigacion Clinica
 Iranian Red Crescent Medical Journal
 JMS - Journal of Medical Society
 Journal of Association of Physicians of India
 Journal of Emergencies Trauma & Shock
 Journal of Infection
 Journal of Medical Sciences
 Journal of Medical Virology
 Journal of Nippon Medical School
 Journal of the Indian Medical Association
 Journal of the Medical Association of Thailand 2009
 Journal of the Royal Army Medical Corps
 Journal of Travel Medicine
 Journal of Tropical Paediatrics
 Kathmandu University Medical Journal
 Kobe Journal of Medical Sciences
 Medicine
 Military Medicine
 Mymensingh medical journal
 National Medical Journal of India
 New England Journal of Medicine
 Pediatric Radiology, Conference

Revista Brasileira de Medicina
 Revista da Associacao Medica Brasileira
 Revista da Sociedade Brasileira de Medicina Tropical
 Revista do Instituto de Medicina Tropical
 Saudi Medical Journal
 Society of Paediatric Radiology
 Southeast Asian Journal of Tropical Medicine and Public Health
 Tehran University Medical Journal
 The American journal of tropical medicine and hygiene
 The Journal of the Association of Physicians of India
 The Medical journal of Malaysia
 The Southeast Asian journal of tropical medicine and public health
 Transactions of the Royal Society of Tropical Medicine & Hygiene
 Travel Medicine and Infectious Disease
 Tropical Doctor
 Tropical Medicine and International Health, Conference
 Tunisie Medicale

General Life Sciences Journal

Acta Tropica
 American Society for Microbiology (ASM),
 Annals of Clinical Microbiology and Antimicrobials
 Annals of the New York Academy of Sciences.
 Antimicrobial Agents and Chemotherapy
 Asian Journal of Microbiology, Biotechnology and Environmental
 Sciences
 Asian Pacific Journal of Tropical Disease
 BMC infectious diseases
 Brazilian Journal of Infectious Diseases
 Clinical and Vaccine Immunology
 Clinical Infectious Diseases
 Clinical Microbiology and Infection, Conference
 Diseases (ICID) Miami, FL United States, Conference Start
 Eastern Mediterranean Health Journal
 Emerging Infectious Diseases
 Epidemiology and Infection
 Harefuah
 Indian Journal of Public Health
 Infection
 Infectious Diseases in Clinical Practice
 International Journal of Antimicrobial Agents
 International Journal of Infectious Diseases

International Journal of Infectious Diseases, Conference
 International Journal of Neuroprotection and Neuroregeneration
 Japanese Journal of Infectious Diseases
 Journal of Clinical Microbiology
 Journal of Global Infectious Diseases
 Journal of Health, Population and Nutrition
 Journal of Infection
 Journal of Infection and Public Health
 Journal of Infection in Developing Countries
 Journal of Infectious Diseases
 Journal of Microbiological Methods
 Journal of the Egyptian Society of Parasitology
 Life Sciences Journals
 Malaria Journal
 Microbiology and Immunology
 Mikrobiyoloji bulteni
 Nephrology Dialysis Transplantation
 Pathogens and Global Health
 PLoS Neglected Tropical Diseases
 PLoS ONE
 Scandinavian Journal of Infectious Diseases
 The Egyptian journal of immunology
 The Scientific World Journal: Immunology
 Ugeskrift for Læger
 Vector-Borne and Zoonotic Diseases

Veterinary Related Journal

Journal of Veterinary Medical Science

Appendix B

List of countries where zoonotic pathogens were reported, and classified by WHO as malaria endemic

Afghanistan
Argentina
Bangladesh
Belize
Bolivia
Brazil
Cambodia
Cameroon
China
Colombia
Costa Rica
Dominican Republic
Ecuador
Ethiopia
Georgia
Ghana
Guinea
Guyana
Haiti
India
Indonesia
Iran
Iraq
Jamaica
Kenya
Laos
Malawi
Malaysia
Mali
Mexico
Morocco
Mozambique
Nepal
Nigeria
Peru
Saudi Arabia
Senegal
Somalia
South Africa
South Korea
Sri Lanka
Sudan

Tanzania
Thailand
Thai-Burmese
Thai-Myanmar
Togo
Tunisia
Turkey
Vietnam.